

STUDY OF METAGENIC EFFECTS OF FD&C RED NO. 2
(71-23) #1

Study of mutagenic effects of FD&C Red #2

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STUDY OF MUTAGENIC EFFECTS OF FD&C RED NO. 2

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A handwritten signature in cursive ink, appearing to read "W. A. Skinner".

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INTRODUCTION

Under contract to the Food and Drug Administration, Stanford Research Institute is examining the mutagenicity of 14 selected chemical compounds (Contract No. FDA 71-267). This report describes the results of tests conducted on the first compound, FD&C Red No. 2 (FDA No. 71-23). Included in this report are detailed descriptions of the methodologies used to perform these tests. The methodologies will not be noted in future reports unless significant changes are made in the experimental procedures.

Three methods are used for evaluating the genetic hazards of the test compounds. These are: (1) Host-Mediated Assay, (2) Cytogenetic Assay, and (3) Dominant Lethal Gene Test. Each procedure is described in detail below.

For the compound under consideration in this report--and as will be the case for the remaining 13 compounds--single and repeated oral administrations were performed at three concentrations. These amounts were (1) a maximum tolerated dose or 5 g/kg (whichever was lower), (2) a low dose of 30 mg/kg or one near the use level, and (3) a level intermediate between the use level and the maximum tolerated dose.

SUMMARY

Host-Mediated Assay

FD&C Red No. 2 was shown to have a mutagenic response in the host-mediated assay for the two strains of Salmonella typhimurium G-46 and TA-1530, particularly when the compound was administered in a chronic or subacute manner. Apparently this mutagenic response is induced by some metabolite rather than by the compound itself, since in vitro exposure of the compound to the bacteria did not induce mutations.

Cytogenetic Studies

In the cytogenetic analysis, FD&C Red No. 2 shows marginal to no responses. With the acute treatment of the very high dose of 5 g/kg, 2.8% of the bone marrow cells examined had an aberration 6 hours after feeding the rats. This is in comparison with 0.7% for the negative control and 13.1% for the positive control, which received an intra-peritoneal injection of 0.5 mg/kg of triethylene melamine. On a subacute basis, the low level (30 mg/kg) showed 2.0% aberrant cells, while the negative controls had 0%. The higher concentrations of FD&C Red No. 2 administered on a subacute basis had a lower percentage of aberrant cells (on a daily dose level, 1.8% for 2.5 g/kg and 0.8% for 5 g/kg).

Dominant Lethal Gene Test

No consistent responses occurred to suggest that FD&C Red No. 2 is mutagenic to the rat as a result of this experimental procedure. The positive reference control compound, TEM, produced mutagenic responses from the second through the fifth weeks of the experiment, as would be expected from this known mutagen.

HOST-MEDIATED ASSAY

Introduction

The development of the host-mediated assay has put into use the advantages of both a microbial system and a mammalian organism in evaluating mutagenesis. With the microbial system, specific mutation frequencies can be determined with relative ease because of the ability to work with large populations that are easy to manipulate. In addition, specific types of mutation can be detected (e.g., point mutation versus frame-shift mutation). The mammalian organism supplies the metabolic activity by which a test compound can be activated or deactivated such as by reduction or hydroxylation.

In the host-mediated assay, the indicator microorganism is injected into the host's peritoneal cavity at the same time the host receives the test compound by some other route, such as oral intubation or intramuscular injection. The microorganism is allowed to incubate while the animal has an opportunity to metabolize the compound. After the organism has had a chance to incubate, it is removed from the animal and is assayed for mutations. Theoretically, during the incubation period, the organism is then exposed to whatever metabolite normally might reach the various tissues in the animal. By comparing the mutagenicity of the compound in vitro with that obtained in the host-mediated assay, it is possible to determine if any activation or deactivation of the test compound has occurred during metabolism in the animal.

This report describes the results obtained from the host-mediated assay as well as the in vitro mutagenic tests for compound 71-23. For the initial report, a detailed description of the methodology has been provided even though it has been generally outlined in the literature (e.g., E. Zeiger and D. Brusick. 1971. The host-mediated assay--a protocol for Salmonella and Saccharomyces. Newsletter of the Environmental Mutagen Society, No. 5, 32-34).

Materials and Methods

Organisms

Two strains of the bacteria Salmonella typhimurium, which are histidine auxotrophs, were used in these studies to measure for biochemical reversion mutations. S. typhimurium G-46 is a his-, missense auxotroph, and S. typhimurium TA-1530 is a G-46 strain containing a gal-bio-uvr B deletion. The yeast Saccharomyces cerevisiae D-3, which is a diploid organism heterozygous for two linked genes (ade 2 and his 8), was used

to measure for mitotic recombination. The strains used were supplied by Dr. Marvin Legator, Food and Drug Administration.

Animals

Male Swiss albino mice, weighing an average of 28-30 gm, were used and maintained on a diet of Purina Lab Chow. The mice were obtained from Horton Laboratories, Inc., Oakland, California.

Preparation of Microorganisms for Inoculation

The Salmonella strains were maintained on tryptone-yeast extract agar slants. To prepare the organism for inoculation into mice, a small inoculum from an agar slant was added to a broth consisting of 1.0% tryptone and 0.5% yeast extract. This culture was incubated for 24 hr at 37°C. The resulting suspension of cells was then adjusted to a concentration of $3-5 \times 10^8$ viable cells/ml using a spectrophotometer.

The yeast strain was maintained on yeast extract (0.5%) glucose (5.0%) agar slants. To prepare the yeast for inoculation into mice, a small inoculum from the agar slant was added to a broth consisting of 5% glucose, 0.5% yeast extract, and 0.2% peptone. This culture was incubated on a rotary shaker at 30°C for 24 hr. The cell concentration was adjusted spectrophotometrically to a concentration of $1-3 \times 10^8$ viable cells/ml before inoculating the animals.

Inoculation of the Mice

Two ml of the appropriate organism were inoculated into the peritoneal cavities of the mice using a 23-gauge needle. The area of inoculation was washed with ethanol before injection. The test compound was administered simultaneously with the inoculation.

Administration of Test Compound

The test compounds were administered by oral intubation using an 18-gauge intubating needle. The compound was dissolved or suspended in Mazola pure corn oil to a concentration requiring a 2.0 ml volume for each intubation.

The positive control compound for Salmonella, dimethyl nitrosamine (DMNA), was dissolved in 10% ethanol to a concentration that would provide a 30-gm mouse with a dose of 100 mg/kg. The positive control for the yeast, ethyl methane sulfonate (EMS), was dissolved in sterile saline to give a dose of 350 mg/kg per mouse. Positive control compounds were administered in 0.10 ml volumes by intramuscular injection.

Negative controls were run in all experiments. The negative control consisted of administering the solvent used for the test compound by oral intubation.

Autopsy and Recovery of Organisms

All test groups were sacrificed 4 hr after inoculation of the organism and administration of the test compound. The mice were sacrificed by cervical dislocation, their exterior abdominal regions were washed with ethanol, and 2 ml of sterile saline were injected into the peritoneal cavity of each mouse. The peritoneal cavity was opened aseptically, and the exudate withdrawn using a tuberculin syringe without a needle. The peritoneal exudates from each mouse were treated individually. They were placed in sterile tubes and immediately put in an ice bath. All plating of the samples was begun immediately after removal from the mouse.

Enumeration of Total Viable and Mutant Cells

Tenfold serial dilutions were made for each peritoneal exudate by serially adding 0.5 ml of sample to 4.5 ml of sterile saline. For the bacteria, a concentration series from 10^0 to 10^{-7} was prepared and for the yeast a series from 10^0 to 10^{-5} .

To enumerate the total viable bacteria, the 10^{-6} and 10^{-7} dilutions were plated by adding 0.2 ml of sample/plate to 3 separate plates. Each sample was spread over the surface of the plate using a sterile, bent glass rod. The medium used to enumerate total viable cells was as follows:

Bacteria Complete Medium

Tryptone	1.0%
Yeast extract	0.5%
Agar	2.0%
Dist. H ₂ O	to desired volume

To enumerate the revertant mutant bacterial cells, the 10^0 (and the 10^{-1} dilution if a large number of revertants were expected) dilutions were plated as described for enumerating the total bacteria. Six plates were used for each sample. The medium used for enumerating mutants was as follows:

Bacteria Minimal Medium

(NH ₄) ₂ SO ₄	0.2%
K ₂ HPO ₄	1.4%
KH ₂ PO ₄	0.6%
Na citrate	0.1%
MgSO ₄	0.02%
Biotin	0.5 µg/ml
Glucose	0.5%
Agar	2.5%
Dist. H ₂ O	to volume

The glucose and biotin were sterilized separately and added to the autoclaved salt solution.

All bacteria were incubated at 37°C; the bacteria complete plates for 18 hr and the bacteria minimal for 40 hr. If the plates could not be counted at this time, they were refrigerated.

To enumerate the yeast (both total viable cells and mitotic recombinants), samples from the 10⁻² to 10⁻⁵ dilutions were plated on a yeast complete medium. They were plated in the same manner as described for the enumeration of the total bacteria. Total viable counts were usually obtained by counting the 10⁻⁵ or 10⁻⁴ plates. The number of mitotic recombinant colonies was usually obtained by scanning the 10⁻³ or 10⁻² plates with a dissecting scope at 10 X. The mitotic recombinants were seen as either red colonies or as red sectors on a normally white yeast colony. The prominence of the mitotic recombinants was enhanced by refrigerating for several days following the normal incubation of the yeast at 30°C for 48 hr.

The medium used for plating yeast was as follows:

Yeast Complete Medium

Yeast extract	0.5%
Peptone	0.35%
Glucose	2.0%
Agar	2.5%
KH ₂ PO ₄	0.15%
MgSO ₄ ·7H ₂ O	0.05%
(NH ₄) ₂ SO ₄	0.45%
Dist. H ₂ O	to desired volume

Data Handling

The data from all mice were used unless a great deal of contamination occurred or low recovery rates were obtained, possibly because the organism might have been injected into some organ rather than the peritoneal cavity. The number of colony forming units (CFU) or mitotic recombinants was determined by:

$$\frac{\text{No. CFU/plate}}{\text{No. plates}} \times \frac{1}{0.2} \times \frac{1}{\text{dilution factor}} =$$

(e.g. $10^0 - 10^{-7}$)

CFU/ml in undiluted exudate

The mutation frequency (MF) was calculated by:

$$MF = \frac{\text{total mutant cells}}{\text{total population}}$$

The relationship between the mutation or recombination frequency of any given treatment (MF_t) to the negative control (MF_c) was also determined as follows:

$$MF_t/MF_c$$

Treatment Groups

All treatment groups, including the positive and negative controls, consisted of 10 mice. The method used to determine concentrations of test compound is described in the section on the Dominant Lethal Gene.

The following groups were tested for all three organisms.

Group	Treatment	Day of Treatment on which Test Organism Was Injected
1	Maximum tolerated dose	1
2	Intermediate dose	1
3	Low dose	1
4	Appropriate positive control	1
5	Appropriate negative control	1
6	Maximum tolerated dose	5
7	Intermediate dose	5
8	Low dose	5
9	Appropriate negative control	5

For testing compound 71-23, the following doses were used:

- Maximum tolerated dose - 5 g/kg
- Intermediate dose - 2.5 g/kg
- Low dose - 30 mg/kg

In Vitro Tests

The method described by Ames was used to determine in vitro mutagenicity for the bacteria (Ames, B. 1971. The detection of chemical mutagens with enteric bacteria, Vol. I, pp. 267-282. In A. Hollaender (ed.), Chemical Mutagens: Principles and Methods for their Detection. Plenum Press, New York).

To determine the in vitro mitotic recombination frequency of the test compound on the yeast, it was first necessary to determine what level of the test compound gave a 50% survival of the organism after a 4-hr exposure at 30°C. If the compound showed no lethal effects, a concentration of 5.0% w/v was used. In the actual test for mitotic recombination, the yeast (approximately 5×10^7 cells/ml) was exposed to the appropriate concentration of compound for 4 hr, and then samples were plated as described for determining mitotic recombinants in the host-mediated assay study. The mitotic recombination frequency is expressed as sectors per 10^5 survivors. This was compared with a negative control.

In both the bacterial and the yeast in vitro studies, EMS was employed as the positive control.

Results and Discussion

Table 1 presents a summary of the host-mediated assay results for compound 71-23. Table 2 contains the data obtained on each individual mouse. This table is a computer printout of the calculations made on the data obtained for each mouse. It was possible to obtain usable data on at least 7 mice in each treatment group in all but one case, which was the negative control acute for S. cerevisiae D-3.

Data on individual mice were excluded only when major contamination or lack of recovery occurred. In one or two cases, an individual mouse produced an exceptionally high mutation frequency. Table 3 summarizes the data obtained in the in vitro assays.

As can be seen in the summary data of Table 1, the test compound shows definite mutagenic responses for the two S. typhimurium strains tested, particularly in the chronic or subacute studies. The mitotic recombination frequency of S. cerevisiae was also increased slightly.

It is rather striking that S. typhimurium G-46 was not greatly responsive in the acute treatment. The MF of the positive control was only 6.84 times as great as the MF of the negative control, and the responses for the test compounds were very different from those of TA-1530. If, however, the data for mouse number 5, which had an unusually high mutation frequency, are excluded from the averages, the results change significantly as follows:

<u>Treatment</u>	<u>MF</u>	<u>Mft/MFc</u>
Maximum	7.63×10^{-7}	24.22
Intermediate	1.31×10^{-7}	4.16
Low	1.23×10^{-7}	3.91
Control (+)	2.16×10^{-6}	68.60
Control (-)	3.15×10^{-8}	1.00

The positive control response of 68.60 using data in which mouse 5 is excluded is more consistent with the Mft/MFc normally obtained. On this basis, it would seem justified to exclude the data for mouse 5.

The lack of mutagenicity of 71-23 in the in vitro tests indicates that it is a metabolite, not the parent compound, that is mutagenic.

Table 1

HOST-MEDIATED ASSAY
SUMMARY OF DATA

Compound No.: 71-23 (FD&C Red No. 2)

A. Acute

Treatment	Organism					
	<u>Salmonella</u>			<u>Saccharomyces</u>		
	G46		TA 1530		D-5	
	MF	MFT/ MFC	MF	MFT/ MFC	RF	RFT/ RFC
Maximum	7.63×10^{-7}	2.41	1.04×10^{-5}	109.35	3.40×10^{-4}	2.25
Intermediate	1.31×10^{-7}	0.41	6.40×10^{-6}	67.29	4.96×10^{-4}	3.28
Low Level	1.23×10^{-7}	0.39	1.60×10^{-6}	16.82	2.85×10^{-4}	1.89
Control (+)	2.16×10^{-6}	6.84	2.34×10^{-6}	24.61	⁽¹⁾ 3.85×10^{-4}	3.89
Control (-)	3.16×10^{-7}	1	9.51×10^{-8}	1	1.51×10^{-4}	1
Control (-)					⁽¹⁾ 9.89×10^{-5}	1

(1) Acute positive control was run separately along with a negative control.

B. Subacute

Treatment	Organism					
	<u>Salmonella</u>			<u>Saccharomyces</u>		
	G46		TA 1530		D-5	
	MF	MFT/ MFC	MF	MFT/ MFC	RF	RFT/ RFC
Maximum	5.87×10^{-4}	438.06	2.27×10^{-5}	908.00	8.22×10^{-5}	2.24
Intermediate	2.88×10^{-5}	21.49	⁽²⁾ 1.04×10^{-5}	75.36	4.08×10^{-5}	1.11
Low Level	8.54×10^{-6}	6.37	3.26×10^{-8}	1.30	5.10×10^{-5}	1.40
Control (-)	1.34×10^{-6}	1	2.50×10^{-8}	1	3.67×10^{-5}	1
Control (-)			⁽²⁾ 1.38×10^{-7}	1		

(2) Intermediate concentration subacute was run separately along with a negative control.

Table 2

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red No. 2)Organism: D-3Treatment: CONTROL (+)

A. Acute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.272222222222ex 05	.688333333330ex 08	.395480225990ex-03
2	.170000000000ex 05	.748333333330ex 08	.227171492205ex-03
3	.188888838888ex 05	.134166666666ex 09	.140786749482ex-03
4	.222222222222ex 05	.116833333333ex 09	.190204469305ex-03
5	.305000000000ex 05	.476666666666ex 08	.639860139861ex-03
6	.355000000000ex 05	.580000000000ex 08	.612068965517ex-03
7	.340000000000ex 05	.561666666665ex 08	.605341246292ex-03
8	.515000000000ex 05	.165833333333ex 09	.310552763819ex-03
9	.275000000000ex 05	.808333333330ex 08	.340206185568ex-03
.384630248723ex-03			

B. Subacute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATA

Compound No.: 71-23 (FD&C Red No. 2)

Organism: D-3

Treatment: CONTROL (-) For Control (+)

A. Acute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.20000000000ex 04	.48000000000ex 08	.41666666666ex-04
2	.31250000000ex 04	.45833333333ex 08	.681818181818ex-04
3	.30000000000ex 04	.30833333333ex 08	.972972972974ex-04
4	.20000000000ex 04	.30333333333ex 08	.659340659341ex-04
5	.22222222222ex 04	.22166666666ex 08	.100250626566ex-03
6	.45000000000ex 04	.40833333333ex 08	.110204081632ex-03
7	.40000000000ex 04	.20166666666ex 08	.198347107438ex-03
8	.45000000000ex 04	.41166666666ex 08	.109311740890ex-03
			.988991755756ex-04

B. Subacute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		

Table 2 (continued)

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red No. 2)Organism: D-3Treatment: CONTROL(-)

A. Acute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml	Forming Units/ml	
1	.25000000000ex 04	.23000000000ex 08	.108695652173ex-03
2	.16666666666ex 03	.83833333330ex 06	.198807157057ex-03
3	.25000000000ex 04	.23475000000ex 08	.106496272630ex-03
4	.45000000000ex 04	.23875000000ex 08	.188481675392ex-03
5	.25000000000ex 04	.13000000000ex 08	.192307692307ex-03
6	.15000000000ex 04	.13250000000ex 08	.113207547169ex-03
			.151332666121ex-03

B. Subacute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml	Forming Units/ml	
1	.10000000000ex 04	.96500000000ex 08	.103626943005ex-04
2	.10000000000ex 04	.53750000000ex 08	.186046511627ex-04
3	.50000000000ex 03	.67500000000ex 08	.740740740740ex-05
4	.10000000000ex 04	.53000000000ex 08	.188679245283ex-04
5	.25000000000ex 04	.27750000000ex 08	.900900900900ex-04
6	.15000000000ex 04	.49000000000ex 08	.306122448979ex-04
7	.20000000000ex 04	.40000000000ex 08	.500000000000ex-04
8	.16666666666ex 04	.21500000000ex 08	.775193798446ex-04
9	.15000000000ex 04	.56750000000ex 08	.264317180616ex-04
			.366551233655ex-04

Table 2 (continued)

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATA

Compound No.: 71-23 (FD&C Red No. 2)

Organism: D-3

Treatment: Low

A. Acute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.40000000000ex 04	.31750000000ex 08	.125984251968ex-03
2	.50000000000ex 04	.37500000000ex 08	.133333333333ex-03
3	.66666666665ex 03	.16000000000ex 07	.416666666665ex-03
4	.20000000000ex 04	.23750000000ex 08	.842105263157ex-04
5	.35000000000ex 04	.24525000000ex 08	.142711518858ex-03
6	.15000000000ex 04	.15300000000ex 08	.980392156862ex-04
7	.37500000000ex 04	.32500000000ex 07	.115384615384ex-02
8	.20000000000ex 04	.75750000000ex 07	.264026402640ex-03
9	.35000000000ex 04	.24600000000ex 08	.142276422764ex-03
			.284566054673ex-03

B. Subacute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.30000000000ex 04	.62250000000ex 08	.481927710843ex-04
2	.20000000000ex 04	.40500000000ex 08	.493827160493ex-04
3	.25000000000ex 04	.69750000000ex 08	.358422939068ex-04
4	.30000000000ex 04	.63500000000ex 08	.472440944881ex-04
5	.50000000000ex 03	.55000000000ex 07	.909090909090ex-04
6	.10000000000ex 04	.22750000000ex 08	.439560439560ex-04
7	.45000000000ex 04	.58000000000ex 08	.775862068965ex-04
8	.25000000000ex 04	.40250000000ex 08	.621118012422ex-04
9	.12500000000ex 04	.47250000000ex 08	.264550264550ex-04
10	.20000000000ex 04	.69750000000ex 08	.286738351254ex-04
			.510353880111ex-04

Table 2 (continued)

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red No. 2)Organism: D-3Treatment: INTERMEDIATE

A. Acute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.15000000000ex 04	.98250000000ex 07	.152671755725ex-03
2	.50000000000ex 03	.70000000000ex 06	.714285714285ex-03
3	.27777777777ex 04	.28250000000ex 08	.983284169122ex-04
4	.33333333333ex 03	.84333333333ex 06	.395256916997ex-03
5	.15000000000ex 04	.69750000000ex 07	.215053763440ex-03
6	.16666666666ex 03	.24500000000ex 06	.680272108840ex-03
7	.20000000000ex 04	.82250000000ex 07	.243161094224ex-03
8	.10000000000ex 04	.19250000000ex 07	.519480519480ex-03
9	.33333333333ex 03	.19166666666ex 06	.173913043478ex-02
10	.10000000000ex 04	.50250000000ex 07	.199004975124ex-03
			.495664569979ex-03

B. Subacute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.50000000000ex 03	.28250000000ex 08	.176991150442ex-04
2	.15000000000ex 04	.66250000000ex 08	.226415094339ex-04
3	.20000000000ex 04	.21000000000ex 08	.952380952380ex-04
4	.10000000000ex 04	.28750000000ex 08	.347826086956ex-04
5	.20000000000ex 04	.60500000000ex 08	.330578512396ex-04
6	.15000000000ex 04	.43750000000ex 08	.342857142857ex-04
7	.15000000000ex 04	.35750000000ex 08	.419580419580ex-04
8	.20000000000ex 04	.43000000000ex 08	.465116279069ex-04
			.407718205075ex-04

Table 2 (continued)

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATA

Compound No.: 71-23 (FD&C Red No. 2)

Organism: D-3

Treatment: MAXIMUM

A. Acute

Mouse No.	Ave. No. Mutant		Mutation or Recombination Frequency
	Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	
1	.30000000000ex 04	.17300000000ex 08	.173410404624ex-03
2	.10000000000ex 04	.28250000000ex 07	.353982300884ex-03
3	.20000000000ex 04	.92750000000ex 07	.215633423180ex-03
4	.33333333333ex 03	.13933333333ex 07	.239234449761ex-03
5	.30000000000ex 04	.14550000000ex 08	.206185567010ex-03
6	.22222222222ex 04	.33250000000ex 07	.668337510442ex-03
7	.50000000000ex 03	.95000000000ex 06	.526315789473ex-03
			.340442777908ex-03

B. Subacute

Mouse No.	Ave. No. Mutant		Mutation or Recombination Frequency
	Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	
1	.30000000000ex 04	.31750000000ex 08	.944881889763ex-04
2	.20000000000ex 04	.17500000000ex 08	.114285714285ex-03
3	.15000000000ex 04	.27500000000ex 08	.545454545454ex-04
4	.20000000000ex 04	.20750000000ex 08	.963855421686ex-04
5	.15000000000ex 04	.26500000000ex 08	.566037735849ex-04
6	.35000000000ex 04	.51000000000ex 08	.686274509803ex-04
7	.25000000000ex 04	.24000000000ex 08	.104166666666ex-03
8	.50000000000ex 03	.73250000000ex 07	.682593856655ex-04
			.821702721086ex-04

Table 2 (continued)

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red No. 2)Organism: G-46Treatment: Control (+)**A. Acute**

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>
1	.984166666665ex 03	.488333333333ex 09	.201535836177ex-05
2	.806666666665ex 03	.129000000000ex 10	.625322997414ex-06
3	.220000000000ex 03	.483333333333ex 08	.455172413793ex-05
4	.570833333330ex 03	.453333333333ex 09	.125919117646ex-05
5	.619166666665ex 03	.795000000000ex 09	.778825995805ex-06
6	.229333333333ex 04	.250500000000ex 10	.915502328674ex-06
7	.211375000000ex 04	.214833333333ex 10	.983902249807ex-06
8	.117000000000ex 04	.250166666666ex 10	.467688207862ex-06
9	.293000000000ex 03	.450000000000ex 08	.65111111111lex-05
10	.294000000000ex 03	.833333333330ex 08	.352800000001ex-05
			.216366265667ex-05

B. Subacute

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>

Table 2 (continued)

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red No. 2)Organism: G-46Treatment: CONTROL (-)

A. Acute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.15000000000ex 02	.101666666666ex 09	.147540983607ex-06
2	.11666666666ex 02	.188333333333ex 10	.619469026546ex-08
3	.50000000000ex 01	.165000000000ex 10	.303030303030ex-08
4	.21666666666ex 02	.107000000000ex 10	.202492211837ex-07
5	.16166666666ex 04	.70000000000ex 09	.230952380951ex-05
6	.33333333333ex 01	.128333333333ex 10	.259740259740ex-08
7	.21566666666ex 02	.144833333333ex 10	.149597238204ex-07
8	.23333333333ex 02	.908333333330ex 09	.256880733945ex-07
			.316223025923ex-06

B. Subacute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.92916666665ex 03	.67500000000ex 09	.137654320987ex-05
2	.12525000000ex 04	.11100000000ex 10	.112837837837ex-05
3	.13175000000ex 04	.10300000000ex 10	.127912621359ex-05
4	.13325000000ex 04	.10566666666ex 10	.126104100947ex-05
5	.12750000000ex 04	.84500000000ex 09	.150887573964ex-05
6	.11091666666ex 04	.17866666666ex 10	.620802238804ex-06
7	.47983333333ex 04	.22166666666ex 10	.216466165414ex-05
8	.38350000000ex 04	.28300000000ex 10	.135512367491ex-05
			.133681901483ex-05

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATA

Compound No.: 71-23 (FD&C Red No. 2)

Organism: G-46

Treatment: Low

A. Acute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.34500000000ex 03	.21833333333ex 10	.158015267175ex-06
2	.10000000000ex 02	.16833333333ex 10	.594059405941ex-08
3	.23333333333ex 02	.16883333333ex 10	.133203356367ex-07
4	.25000000000ex 02	.196666656666ex 10	.127118644063ex-07
5	.28333333333ex 02	.16083333333ex 10	.176165803108ex-07
6	.44166666666ex 03	.48666666666ex 09	.907534246575ex-06
7	.30000000000ex 02	.10283333333ex 10	.291734197731ex-07
8	.26666666666ex 02	.43000000000ex 09	.620155038758ex-07
9	.83333333330ex 01	.92666666665ex 09	.899280575537ex-08
10	.11666666666ex 02	.58166666665ex 09	.200573065902ex-07
			.123587702414ex-06

B. Subacute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.11357500000ex 05	.71166666665ex 09	.159590163934ex-04
2	.84000000000ex 04	.16016666666ex 10	.524453694070ex-05
3	.14315000000ex 05	.71500000000ex 09	.200209790209ex-04
4	.36040000000ex 04	.33333333333ex 09	.10812000000ex-04
5	.15732500000ex 05	.15266666666ex 10	.103051310044ex-04
6	.43200000000ex 04	.15933333333ex 10	.271129707113ex-05
7	.44166666666ex 02	.11466666666ex 10	.385174418606ex-07
8	.56600000000ex 04	.16233333333ex 10	.348665297741ex-05
9	.17795833333ex 05	.21433333333ex 10	.830287713841ex-05
			.854233422090ex-05

Table 2 (continued)

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red No. 2)Organism: G-46Treatment: INTERMEDIATE

A. Acute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.10000000000ex 02	.99333333330ex 09	.100671140939ex-07
2	.11666666666ex 02	.85833333330ex 09	.135922330096ex-07
3	.18333333333ex 02	.88833333330ex 09	.206378986867ex-07
4	.18333333333ex 02	.11533333333ex 10	.158959537572ex-07
5	.25000000000ex 03	.10450000000ex 10	.239234449760ex-06
6	.39000000000ex 03	.54500000000ex 09	.715596330275ex-06
7	.31666666666ex 02	.67333333330ex 09	.470297029704ex-07
8	.16666666666ex 02	.74000000000ex 09	.225225225224ex-07
9	.15000000000ex 02	.68333333330ex 08	.219512195123ex-06
10	.33333333333ex 01	.55166666665ex 09	.604229607251ex-08
			.131013069626ex-06

B. Subacute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.17412500000ex 05	.10233333333ex 10	.170154723127ex-04
2	.15511666666ex 05	.98333333330ex 09	.15774576271lex-04
3	.39165000000ex 05	.54333333330ex 09	.720828220863ex-04
4	.19075000000ex 05	.84166666665ex 09	.226633663366ex-04
5	.11427500000ex 05	.21666666666ex 09	.527423076924ex-04
6	.22555000000ex 05	.29883333333ex 10	.754768544339ex-05
7	.77500000000ex 04	.74000000000ex 09	.104729729729ex-04
8	.40579000000ex 05	.14350000000ex 10	.282780487804ex-04
9	.37852500000ex 05	.11550000000ex 10	.327727272727ex-04
			.288166643516ex-04

Table 2 (continued)

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATA

Compound No.: 71-23 (FD&C Red No. 2)Organism: G-46Treatment: MAXIMUM

A. Acute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.833333333330ex 01	.803333333330ex 09	.103734439834ex-07
2	.833333333330ex 01	.441666666666ex 09	.188679245282ex-07
3	.250000000000ex 02	.134000000000ex 10	.186567164179ex-07
4	.150000000000ex 02	.118333333333ex 10	.126760563380ex-07
5	.183333333333ex 02	.401666666666ex 09	.456431535269ex-07
6	.153333333333ex 04	.296666666666ex 09	.516853932584ex-05
7	.281666666666ex 03	.430000000000ex 10	.655038759683ex-07
			.762894356655ex-06

B. Subacute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.299425000000ex 05	.833333333330ex 07	.359310000001ex-02
2	.988333333330ex 05	.893333333330ex 09	.110634328358ex-03
3	.269191666666ex 05	.488333333333ex 09	.551245733787ex-04
4	.114833333333ex 04	.866666666665ex 08	.132499999999ex-04
5	.294966666666ex 05	.283333333333ex 09	.104105882352ex-03
6	.738850000000ex 05	.376666666666ex 09	.196154867256ex-03
7	.305025000000ex 05	.851666666665ex 09	.358150684932ex-04
			.586883531401ex-03

Table 2 (continued)

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATA

Compound No.: 71-23 (FD&C Red No. 2)

Organism: TA-1530

Treatment: CONTROL(+)

A. Acute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recom- bination Frequency
	Colonies or Recom- binants/ml		
1	.44833333333ex 03	.24666666666ex 09	.181756756757ex-05
2	.11266666666ex 04	.47000000000ex 09	.239716312055ex-05
3	.24156666666ex 04	.58333333330ex 09	.414285714286ex-05
4	.89833333330ex 03	.31500000000ex 09	.285135185184ex-05
5	.19666666666ex 04	.42166666666ex 09	.466403162054ex-05
6	.10416666666ex 04	.11666666666ex 10	.892857142856ex-06
7	.17833333333ex 04	.53610500000ex 12	.332646278868ex-08
8	.10833333333ex 04	.55833333330ex 09	.194029850746ex-05
			.233874417702ex-05

B. Subacute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recom- bination Frequency
	Colonies or Recom- binants/ml		

Table 2 (continued)

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red No. 2)Organism: TA-1530Treatment: CONTROL(-)**A. Acute**

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>
1	.10000000000ex 03	.10283333333ex 10	.972447325773ex-07
2	.73333333330ex 02	.12050000000ex 10	.608575380356ex-07
3	.10000000000ex 03	.16500000000ex 10	.606060606060ex-07
4	.41666666666ex 02	.70666666665ex 09	.589622641509ex-07
5	.46666666666ex 02	.12266666666ex 10	.380434782610ex-07
6	.10166666666ex 03	.11150000000ex 10	.911808669650ex-07
7	.18333333333ex 02	.86666666665ex 08	.211538461538ex-06
8	.31666666666ex 02	.23666666666ex 09	.133802816901ex-06
9	.98333333330ex 02	.94500000000ex 09	.104056437389ex-06
			.951436284913ex-07

B. Subacute

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>
1	.16666666666ex 02	.12033333333ex 10	.138504155124ex-07
2	.31666666666ex 02	.10633333333ex 10	.297805642633ex-07
3	.36666666666ex 02	.17783333333ex 10	.206185567010ex-07
4	.25000000000ex 02	.95666666665ex 09	.261324041812ex-07
5	.23333333333ex 02	.20433333333ex 10	.114192495921ex-07
6	.16666666666ex 02	.26666666666ex 09	.62499999999ex-07
7	.28333333333ex 02	.26416666666ex 10	.107255520504ex-07
			.250038203284ex-07

Table 2 (continued)

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red No. 2)Organism: TA-1530Treatment: CONTROL (-) (for intermediate)

A. Acute

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>
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B. Subacute

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>
1	.18333333333ex 02	.74666666666ex 09	.513392857143ex-07
2	.88333333330ex 02	.11950000000ex 10	.739191073916ex-07
3	.11833333333ex 03	.78333333330ex 09	.151063829787ex-06
4	.68333333330ex 02	.93833333330ex 09	.728241563054ex-07
5	.13333333333ex 02	.73500000000ex 09	.181405895691ex-07
6	.11333333333ex 03	.15416666666ex 10	.735135135136ex-07
7	.11833333333ex 03	.14300000000ex 10	.827505827503ex-07
8	.15666666666ex 02	.31333333333ex 09	.531914893615ex-07
9	.10500000000ex 03	.13715666666ex 10	.765492102069ex-07
10	.22500000000ex 03	.31166666666ex 09	.721925133691ex-06
			.137521689828ex-06

Table 2 (continued)

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red No. 2)Organism: TA-1530Treatment: Low

A. Acute

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>
1	.616666666665ex 02	.112166666666ex 10	.549777117386ex-07
2	.185000000000ex 03	.560000000000ex 09	.330357142857ex-06
3	.816666666665ex 02	.122833333333ex 10	.664857530529ex-07
4	.156333333333ex 05	.103166666666ex 10	.151534733441ex-04
5	.683333333330ex 02	.130333333333ex 10	.524296675190ex-07
6	.583333333330ex 02	.102500000000ex 10	.569105691053ex-07
7	.450000000000ex 02	.275000000000ex 09	.163636363636ex-06
8	.600000000000ex 02	.991666666665ex 09	.605042016807ex-07
9	.583333333330ex 02	.149166666666ex 10	.391061452513ex-07
10	.533333333330ex 02	.123000000000ex 10	.433604336040ex-07
			.160212413323ex-05

B. Subacute

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>
1	.566666666665ex 02	.262000000000ex 10	.216284987276ex-07
2	.266666666666ex 02	.170000000000ex 09	.156862745097ex-06
3	.216666666666ex 02	.147000000000ex 10	.147392290248ex-07
4	.283333333333ex 02	.215833333333ex 10	.131274131274ex-07
5	.200000000000ex 02	.164833333333ex 10	.121334681496ex-07
6	.116666666666ex 02	.204333333333ex 10	.570962479606ex-08
7	.833333333330ex .01	.192500000000ex 10	.432900432898ex-08
			.326471404640ex-07

Table 2 (continued)

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red No. 2)Organism: TA-1530Treatment: INTERMEDIATE

A. Acute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.46483333333ex 05	.10716666666ex 10	.433748055989ex-04
2	.64000000000ex 03	.91000000000ex 09	.703296703296ex-06
3	.53333333330ex 02	.12733333333ex 10	.418848167537ex-07
4	.11333333333ex 03	.89000000000ex 09	.127340823969ex-06
5	.53333333330ex 02	.14850000000ex 10	.359147025811ex-07
6	.43333333333ex 03	.93333333330ex 09	.464285714287ex-06
7	.20000000000ex 02	.82000000000ex 09	.243902439024ex-07
			.6395983837190ex-05

B. Subacute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.53833333330ex 04	.16333333333ex 09	.329591836733ex-04
2	.18833333333ex 03	.17666666666ex 09	.106603773585ex-05
3	.34166666666ex 03	.27500000000ex 10	.124242424242ex-06
4	.51666666665ex 03	.86500000000ex 09	.597302504815ex-06
5	.37500000000ex 03	.53833333330ex 10	.696594427248ex-07
6	.22000000000ex 04	.63333333330ex 08	.347368421054ex-04
7	.33666666666ex 03	.15500000000ex 10	.217204301074ex-06
8	.19950000000ex 05	.14800000000ex 10	.134797297297ex-04
			.104062752396ex-04

Table 2 (concluded)

HOST-MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red No. 2)Organism: TA-1530Treatment: MAXIMUM

A. Acute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.733333333330ex 02	.800000000000ex 09	.916666666662ex-07
2	.833333333330ex 02	.356666666666ex 09	.233644859812ex-06
3	.700000000000ex 02	.660000000000ex 09	.106060606060ex-06
4	.147333333333ex 05	.571666666665ex 09	.257725947522ex-04
5	.508333333330ex 04	.536666666665ex 09	.947204968940ex-05
6	.416666666666ex 02	.511666666665ex 09	.814332247558ex-07
7	.291666666666ex 04	.533333333330ex 08	.546875000002ex-04
8	.166666666666ex 02	.96666666665ex 09	.172413793103ex-07
9	.155000000000ex 04	.506666666665ex 09	.305921052632ex-05
			.103912668560ex-04

B. Subacute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.10143333333ex 06	.83333333330ex 09	.121720000000ex-03
2	.683333333330ex 03	.776666666665ex 09	.879828326177ex-06
3	.281666666666ex 03	.642500000000ex 09	.438391699091ex-06
4	.300000000000ex 02	.145833333333ex 10	.205714285714ex-07
5	.230000000000ex 03	.103333333333ex 10	.222580645162ex-06
6	.303666666666ex 05	.520000000000ex 09	.583974358973ex-04
7	.333333333333ex 01	.143000000000ex 10	.233100233100ex-08
8	.150000000000ex 02	.104166666666ex 10	.144000000000ex-07
			.227119423746ex-04

Table 3

HOST-MEDIATED ASSAY
IN VITRO MUTAGENICITY OF COMPOUND 71-23 (FD&C RED NO. 2)

Salmonella typhimurium G-46

5% w/v 71-23 EMS

negative positive

Salmonella typhimurium TA-1530

5% w/v 71-23 EMS

negative positive

Saccharomyces cerevisiae D-3

Compound	Concentration	Survival (%)	Recombinants/10 ⁵	
			Survivors	
71-23	5% w/v	142%		8.65
EMS	1% w/v	2.02%		1.07 X 10 ³
Control (-)	--	100%		6.15

CYTOGENETIC ASSAY

Introduction

Of the various methods available for mutagenicity testing, cytogenetic analysis is one of the most widely used. To date, metaphase chromosomes have provided the classic assay system, because it allows the investigator not only to identify and distinguish the numerous types of chromosomal abnormalities, but also, by varying the cell type and/or the exposure time element, to ascertain at what stage of the cell cycle the agent in question is having its effect: before DNA synthesis in G₁ or subsequent to it in G₂. Both reproductive (e.g., spermatocytes) and somatic (e.g., bone marrow) cells can be useful in this regard, each type, of course, giving an indication of possible alternative effects of the compound being tested.

More recently, analysis of anaphase chromosomes has been suggested as a feasible supplementary or alternative method for the rapid scanning of cytogenetic damage caused by test compounds. Although the method does not allow the detailed analysis of types of chromosome damage extractable from metaphase observations, it is much more rapid, both in preparation of in vitro cultures and in scoring, and quickly reveals gross chromosomal damage induced by breaks and interchanges.

Thus, the information gained by cytogenetic analysis of anaphase chromosomes, supported by the more specific information gained by analysis of metaphase chromosomes, when considered together with data from other mutagenic assay systems provides substantial evidence for the mutagenic potential of the compound in question.

Materials and Methods

Animals

To evaluate the mutagenic potential of food additives on the GRAS list, in vivo cytogenetic analysis of rat bone marrow cells in metaphase was employed. Random bred, male, albino rats, 7.5 weeks of age, weighing approximately 200 grams, were obtained from Simonsen Laboratories, Gilroy, California, several days before the start of testing. Thirteen groups were housed five per cage, and four groups were housed three per cage, each cage representing one test group.

Administration of Test Compound and Treatment Groups

FDA Compound 71-23 was suspended in Mazola pure corn oil in the following concentrations:

- (1) 5 g/kg or maximum tolerated dose (MTD) - suspended 1 g compound/4 cc corn oil.
- (2) 2.5 g/kg or intermediate dose (I) - suspended 0.5 g compound/4 cc corn oil.
- (3) 30 mg/kg or low level dose (L) - suspended 6 mg compound/4 cc corn oil.

The compound was kept in suspension during the treatment period by means of magnetic stirrers. Negative controls received corn oil only. Each rat received 4 cc of the appropriate compound administered orally with a 16-gauge intubation needle. Rats were not starved before intubation. Positive controls were injected with 1 cc intraperitoneally of a 0.5 mg/kg triethylene melamine (TEM) solution (dissolved in normal saline).

Individual rats were treated as follows:

<u>Dose</u>	<u>No. Animals</u>
Maximum tolerated dose	15
Intermediate dose	15
Low level dose	15
Corn oil only	9
TEM at 0.5 mg/kg	5

Six hours subsequent to intubation, five rats each from the MTD, I, and L groups; three rats from the Negative group; and all five rats of the Positive group were sacrificed, and bone marrow was obtained. An additional five from each of the MTD, I, and L groups, and three from the Negative group, were sacrificed again at 24 hours and at 48 hours, and bone marrow was obtained.

In conjunction with this single administration acute study, a subacute study was undertaken simultaneously by administering compound every 24 hours for five days as follows:

<u>Dose</u>	<u>No. Animals</u>
Maximum tolerated dose	5
Intermediate dose	5
Low level dose	5
Corn oil only	3

All rats in the subacute study were sacrificed six hours after the last intubation, and bone marrow was obtained.

Preparation of Bone Marrow Cells

To obtain a high population of cells in metaphase, rats were injected with a 4 mg/kg dose of colcemid (CIBA) 75 minutes before sacrifice. (It was found that injection of colcemid two hours before sacrifice caused extreme shortening of chromosomes, making analysis difficult and unreliable.) Bone marrow was obtained by sacrificing rats in a CO₂ atmosphere, removing one femur from each rat by dislocating it at the knee and hip, removing as much of the adherent muscle as possible, cutting off the femoral head, expressing the bone marrow through the orifice thus exposed by means of Eagle's S-MEM contained in a syringe and 21-gauge needle inserted into the end of the femur proximal to the knee, and flushing several times. The marrow was suspended in a total of 5 cc Eagle's S-MEM, with care being taken to obtain a homogeneous suspension free of clumps. Cells were then centrifuged in a clinical centrifuge at approximately 2,500 rpm for 3 minutes, the supernatant aspirated completely, and the cells resuspended thoroughly in 4.5 cc of 0.75% sodium citrate, covered, and incubated for 15 minutes at 37°C with occasional vigorous agitation. Subsequent to incubation, cells were centrifuged (~1200 rpm) for 6 minutes, supernatant and debris aspirated, and 3 cc cold fresh fixative (3:1 methanol: acetic acid) were added without disturbing the cell button. The tubes were stoppered and left at room temperature for 45 minutes, after which all but 3/4 cc of the supernatant was removed, and the cells were resuspended. Volume of fixative was then increased to 3 cc in each tube, and the tubes were stoppered and left overnight at 4°C before spreading.

Preparation of Slides

Before making slides, the cells were centrifuged (~1700 rpm) for 3 minutes, all old fixative was aspirated, 1.5 cc of fresh fixative was added, and the cells were resuspended. The cells were then spread evenly on thoroughly cold and wet slides; the slides were blown on vigorously until nearly dry, at which time they were gently flamed until most of the fixative had evaporated.

Slides were stained in aceto-orcein (1% natural orcein in 60% acetic acid) for 1 hour, rinsed twice in 95% ethanol, once in 100% ethanol, and cleared for 1 hour in xylene before mounting permanently with Permount.

In vitro Tests

For in vitro cytogenetic testing involving anaphase analysis, diploid human embryonic lung cells (WI-38) were obtained from Microbiological Associates, Albany, California.

Cells were received in a confluent state in roller drum tubes. The medium was removed, and 5 ml of varying dilutions of Compound 71-23 completely dissolved in Eagle's minimal essential medium containing Earle's salts (S-MEM)--supplemented with 2% fetal calf serum, 1% glutamine, and 1% pen-strep--were added to each tube. Doubling dilutions of Compound 71-23 ranged as follows: 1000, 500, 250, 125, 62.5, ~31, ~16, ~8, ~4, ~2, and ~1 $\mu\text{g}/\text{ml}$. All doses were run in triplicate. None of the doses was cytotoxic.

After determination of the cytotoxic dose, WI-38 cells, 25% confluent on 11 X 22 mm coverslips in Leighton tubes, were received from the same supplier. Two ml aliquots of the MTD (1,000 $\mu\text{g}/\text{ml}$ for 71-23), as well as doses 10 and 100 times less (100 and 10 $\mu\text{g}/\text{ml}$, respectively), dissolved in the medium used in the cytotoxicity studies (with the exception that serum concentration was increased to 10%) were added to the tubes. TEM at concentrations of 0.5 $\mu\text{g}/\text{ml}$ and 0.1 $\mu\text{g}/\text{ml}$, which had no cytotoxic effects when tested previously, also dissolved in the same media was used as the positive control. Negative control tubes received supplemented medium only. All doses were again run in triplicate. After 24 hours, the medium containing the test compound was removed and fresh medium (10% serum without test compound) added to the cells to ensure continued mitoses. Cells were lightly gassed (95% air, 5% CO_2), to adjust pH, and culturing continued an additional 24 hours before fixing.

Fixing and staining of cells on coverslips was accomplished directly in the Leighton tubes. Media was removed, and cells were rinsed in absolute methanol after which they were fixed for 30 minutes (3:1 methanol: acetic acid). Subsequent to preservation, fixative was removed, and cells were rinsed in 70% methanol, 30% methanol, and water consecutively and then allowed to air dry. The staining procedure was the same as that used for metaphase (bone marrow) cells. After clearing in xylene, coverslips were removed from the Leighton tubes and mounted permanently with Permount.

Reading of Slides

Permanent slides were divided into two identical groups (one slide/rat in the in vivo study and three slides per treatment in the in vitro study), each group separately mixed at random and coded by an individual not involved in reading and scoring. No individual involved in scoring therefore knew which slide was being read, nor could comparisons be made between similar code numbers in each group. Slides were not decoded until all slides in each group were read completely.

Results and Discussion

Table 4 summarizes metaphase data grouped according to times of sacrifice and thus maximal effective treatment period. It compares the effect of varying the dose within any particular time period. Table 5 contains the same information but grouped according to dosage levels. It allows a comparison of effect of time on the chromosomal morphology of cells treated with any particular dose. Table 6 summarizes the results of anaphase analysis.

Before scrutinizing the results of the metaphase data further, two facts should be noted: (1) presence of fragments in cells, even if breaks could not be observed, were taken as evidence of breakage, and (2) cells scored as having greater than 10 aberrations per cell were also considered as evidence for the occurrence of breakage. Both of these types of aberrations are thus included in the data under the heading "Percent Cells with Breaks."

Considering first the percentage of cells with aberrations, as shown in Table 4, it should be noted that at 6 hours the low and intermediate level doses show no effect, while the MTD exhibits a slight response of 2.8% compared with a 0.7% negative control but a 13.1% positive control. This increase is due mainly to an increase in the number of cells with breaks. At 24 hours, no effect is noted at the low level dose, while slightly increasing effects are to be observed at the intermediate and maximum levels. Again, these increases are due to increases in numbers of cells with breaks. At 48 hours, no effect is to be observed at any dosage level. Data for subacute treatment indicate a reverse trend, although with only a minor effect. Cells show an initial increase in aberrations at the low level dose, which declines steadily at the two higher dosages. Although the higher percentage of aberrant cells at low level dose is the result of an increased breakage rate, the percentage of aberrant cells at the intermediate and maximum doses is the result of increased rearrangements as well. At 2,500 mg/kg (intermediate dose), the percentage of cells with more than one type of aberration reaches the level of the positive control. However, this value is so low that it is probably insignificant.

In essence, then, Compound 71-23 exhibits a slight effect at only the highest dosage at 6 hours, tapering off slightly at 24 hours, and becoming nonexistent at 48 hours. Effects are due to increased chromosomal breakage. In the subacute study, slight effects are noted only at the two lower doses and are the combined result of increased breakage and rearrangements. Also, although there is a slight decrease in the mitotic index at 24 hours, no basis appears for attributing a delayed adverse effect on mitoses or chromosomal morphology to Compound 71-23.

Brief consideration of Table 5 indicates that length of exposure to each dose level results in individual trends that depend on the dose itself. The negative control (corn oil only) exhibits no variation with respect to time, while the low and intermediate level doses show a direct

Table 4
CYTOGENETIC ASSAY
METAPHASE SUMMARY SHEET BY TIME OF SACRIFICE
Compound 71-23

<u>Dosage (mg/kg)</u>	<u>Time*</u>	<u>Mitotic Index (%)</u>	<u>No. of Animals</u>	<u>No. of Cells</u>	<u>Cells with Breaks (%)</u>	<u>Cells with Rearrange-ments (%)</u>	<u>Cells with More than one Type of Aber. (%)</u>	<u>Cells with Aber. (%)</u>
Positive Control TEM (0.5 mg/kg)	6	0.96%	5	245	12.7%	0.8%	0.4%	13.1%
Negative Control (Corn oil only)	6	1.8	3	150	0.7	0	0	0.7
30 mg/kg	6	1.76	5	250	0	0	0	0
2500 mg/kg	6	2.4	5	250	0.8	0	0	0.8
5000 mg/kg	6	2.28	5	250	2.4	0.4	0	2.8
Negative Control	24	1.37	3	150	0	0	0	0
30 mg/kg	24	1.22	5	250	0	0	0	0
2500 mg/kg	24	1.6	5	250	0.8	0	0	0.8
5000 mg/kg	24	2.15	5	213	1.9	0	0	1.9
Negative Control	48	2.37	3	150	0.7	0	0	0.7
30 mg/kg	48	2.48	5	250	1.2	0	0	1.2
2500 mg/kg	48	1.98	5	250	0.8	0	0	0.8
5000 mg/kg	48	2.14	5	250	0.4	0.4	0	0.8
Negative Control	SA**	2.3	3	150	0	0	0	0
30 mg/kg	SA	2.3	5	245	2.0	0	0	2.0
2500 mg/kg	SA	2.06	5	225	1.3	0.9	0.4	1.8
5000 mg/kg	SA	2.64	5	245	0.4	0.4	0	0.8

* Time of sacrifice after treatment (hours).

** SA = subacute.

Table 5
 CYTOGENETIC ASSAY
 METAPHASE SUMMARY SHEET BY DOSE LEVELS
 Compound 71-23

<u>Dosage (mg/kg)</u>	<u>Time*</u>	<u>Mitotic Index (%)</u>	<u>No. of Animals</u>	<u>No. of Cells</u>	<u>Cells with Breaks (%)</u>	<u>Cells with Rearrange-ments (%)</u>	<u>Cells with More than one Type of Aber. (%)</u>	<u>Cells with Aber. (%)</u>
Positive Control	6	0.96%	5	245	12.7%	0.8%	0.4%	13.1%
Negative Control	6	1.8	3	150	0.7	0	0	0.7
	24	1.37	3	150	0	0	0	0
	48	2.37	3	150	0.7	0	0	0.7
	SA**	2.3	3	150	0	0	0	0
30 mg/kg	6	1.76	5	250	0	0	0	0
	24	1.22	5	250	0	0	0	0
	48	2.48	5	250	1.2	0	0	1.2
	SA	2.3	5	245	2.0	0	0	2.0
2500 mg/kg	6	2.4	5	250	0.8	0	0	0.8
	24	1.6	5	250	0.8	0	0	0.8
	48	1.98	5	250	0.8	0	0	0.8
	SA	2.06	5	225	1.3	0.9	0.4	1.8
5000 mg/kg	6	2.28	5	250	2.4	0.4	0	2.8
	24	2.15	5	213	1.9	0	0	1.9
	48	2.14	5	250	0.4	0.4	0	0.8
	SA	2.64	5	245	0.4	0.4	0	0.8

* Time of sacrifice after treatment (hours).

** SA = subacute.

Table 6

CYTOGENETIC ASSAY
ANAPHASE SUMMARY SHEET
Compound 71-23

<u>Dosage</u>	<u>Time*</u>	<u>No. of Cells</u>	<u>Cells with Acentric Fragments (%)</u>	<u>Cells with Bridges (%)</u>	<u>Multipolar Cells (%)</u>	<u>Cells with More than One Type Aber. (%)</u>	<u>Cells with Aber. (%)</u>
Negative Control	48	83	12.0%	6.0%	--%	2.4%	15.7%
10 µg/ml	48	132	6.1	14.4	0.8	3.0	18.2
100 µg/ml	48	117	15.4	22.2	--	9.4	28.2
1000 µg/ml	48	161	5.6	13.0	--	1.9	16.8
Positive Control (TEM @ 0.5 µg/ml and 0.1 µg/ml)	48		No anaphases at either concentration				

* Time of harvest after treatment (hours).

effect, causing an increased number of aberrations with increased exposure time. Conversely, the maximum dose exhibits an inverse relationship, causing decreased numbers of aberrations with increased exposure time. This latter effect may possibly be attributed to the unphysiological nature of the maximum dose, which is constantly excreted by the animals within several hours after intubation.

Before discussing the anaphase data, one additional note should be made. Although not included in the final summary of aberrant cell percentages, there was a definite indication of increased polyploidy in the 6-, 24-, and 48-hour treatment groups:

	Percent Polyploid Cells (hr)			
	6	24	48	SA
Positive Control	2.4%	--%	--%	--%
Negative Control	0	1.3	1.3	5.3
30 mg/kg	1.6	0.8	3.2	0
2,500 mg/kg	2.4	4.0	2.4	5.3
5,000 mg/kg	1.6	3.8	4.0	1.6

In considering the WI-38 anaphase data contained in Table 6, a preliminary comment should be made regarding the controls. The positive control (TEM at 0.5 and 0.1 µg/ml) slides contained confluent cultures of cells, unlike any of the other slides, and exhibited no anaphase figures whatsoever. Undoubtedly, this absence was the result of contact inhibition exhibited by these cells in the confluent state, and possibly caused by the beneficial influence of a lower pH when the TEM was dissolved in the media. The negative control, on the other hand, appears to exhibit a high degree of aberrant cells (15.7%), whereas control cells should preferably show aberrances in only about 2% of the population. (W. W. Nichols, P. Moorhead, G. Brewen. 1971. Chromosome Methodologies in Mutation Testing. Newsletter of the Environmental Mutagen Society. No. 5, 20-23).

Bearing these facts in mind, analysis of the data contained in Table 6 reveals a notably increased percentage of aberrant cells over that found for negative controls only at the 100 µg/ml dose; the other two doses (10 µg/ml and 1000 µg/ml) did not exhibit any appreciable increase. None of the doses given caused a significant increase in the number of cells with acentric fragments, and, indeed, the lowest and highest dosages given caused noticeable decreases in this type of aberration. On the other hand, all three dosages caused a sharp increase in the percentage of cells exhibiting bridges compared with the controls. Only the 100 µg/ml dose simultaneously exceeded the control level in number of cells with fragments, while sharply increasing the number of cells exhibiting bridges, thus explaining its greater overall effect when compared with the negative control. The increased number of cells showing more than one type of aberration at this dose indicates that at this dose, unlike any other, individual cells were damaged more severely.

In general, it would appear that Compound 71-23 administered in vitro at any of the dosages used causes an increased number of multiple breaks and rearrangements, as attested to by the increased number of bridges, while decreasing or not affecting the number of cells having only acentric fragments, which are the result of single breaks.

DOMINANT LETHAL GENE

Introduction

Dominant lethal assays of compounds suspected of causing major genetic damage in animals have been carried out, for the most part, in mice. One exception was a comparative study by Bateman with mice and rats to evaluate the dominant lethal effect of triethylenemelamine (*Genet. Res. Comb.* 1, 381-392, 1960). Although there are cost savings in using the mouse rather than the rat, the latter has experimental advantages in providing more definitive information when attempting to assess the incidence of early fetal deaths. Also, corpora lutea counts in the mouse are difficult and relatively imprecise (Epstein and Rohrborn, *Nature* 230, 469-470, 1971). For this project, adult Sprague-Dawley-derived rats, from a closed random-bred colony, are used for both the acute toxicity determinations and the dominant lethal assay.

In this mammalian test procedure, the material under investigation is administered orally either once or on five successive days to proven male breeders. Following dosing, each male is mated with two adult female rats for five days. The females are then removed, the males rested for two days, and new females again added for another breeding. This sequence is continued for eight weeks to allow coverage of the entire spermatogenic development cycle. Thus, this procedure emphasizes possible mutagenic effects on the male sperm, with the normal female acting as a carrier to demonstrate any abnormalities that may have occurred in the male. Effects are evaluated by examining the condition and state of fetal development during the middle to latter stages of gestation.

The experimental approach is presented below in a step-by-step manner to ensure clarity and an understanding of the preciseness of procedures used in this phase of the experimental program.

Methods and procedures are presented in the following order: animals, chemical supply, solubility studies, LD₅₀ determinations (both single and multiple treatment), dosage selections, control groups (both positive and vehicle treated), acute studies (single dose), subacute studies (multiple dose), necropsy, observations, and evaluation.

Materials and Methods

Animals

Adult male and female Sprague-Dawley-derived rats were supplied by Simonsen Laboratories, Gilroy, California. The males were proven breeders,

while the females were of virgin stock. Purina Lab Chow and water were available at all times.

Chemical Supply

All compounds or natural materials were supplied by the Food and Drug Administration. Each compound or natural material was provided in a ready-to-use form and was identified by both the name and FDA code number. Sufficient quantities to complete all aspects of the experimental program were received. Excess supplies have been placed in storage should they be needed for future reference.

Solubility Studies

The solubility of each compound or natural material was tested with various agents such as water, propylene glycol, polyethelene glycol, corn oil, tricaprylin, carboxymethylcellulose, or methylcellulose (Methocel) to determine the most appropriate vehicle for administration. Because of the low toxicity of most materials, and the consequent high dosages given, many of the test materials were given as suspensions.

LD₅₀ (Single and Multiple Dose)

Although acute toxicity information on some of the compounds was available in the literature, confirmatory tests were done to obtain an LD₅₀ under the conditions of this laboratory and for this strain of rats. If no data were available, a broad range-finding dose regimen was initially conducted, followed by an accurate determination of the oral LD₅₀.

To determine a multiple dose LD₅₀, a range-finding dose regimen was established using the acute LD₅₀ data, followed by an accurate determination of the multiple dose LD₅₀. Nonstarved animals were used throughout, because of the multiple dosing regimen.

Dosage Selection

In selecting dose levels for the experimental study, two approaches were used:

- (1) If a finite LD₅₀ was obtained on a compound, the highest dose level was 80% of the calculated LD₅₀. The lowest dose was an overall "low" level--a mutually defined value (FDA and SRI) of 2.1 g per day of the GRAS material for a 70 kg man, equivalent to 30 mg/kg. The intermediate dose was approximately midway between these extremes.

- (2) If the LD₅₀ was greater than 10 g/kg (a mutually agreed on upper limit), the highest dose was 5 g/kg. The lowest dose was the above-defined "low" level, 30 mg/kg. The intermediate dose was approximately midway between these two extremes; we chose 2.5 g/kg. These guidelines were used for both single and multiple dose experimental study groups.

Control Groups (Both Vehicle and Positive)

A vehicle control group (corn oil, water, Methocel, etc.) was included in each experimental study. Vehicle control animals were treated for five consecutive days before the initiation of a particular experiment. In this manner, eight weeks of implant data were obtained for each vehicle control and were used as reference comparisons for the experimentally treated animals, both single and multiple treatment groups. The positive reference control was the known mutagen, triethylene melamine (TEM), given at a dose of 0.2 mg/kg as a single ip injection. Breeding and implant data were obtained for eight weeks.

Acute Studies (Single Dose)

In an acute study, ten experienced male rats per treatment group were administered orally a single dose of test material. Controls were treated as previously described. Within two to three hours, each male was mated with two virgin females of breeding age for a period of five days. Females were replaced weekly for a total mating period of eight weeks.

Subacute Studies (Multiple Dose)

For the subacute assay, the experimental parameters used in the acute test were employed, with three exceptions: (1) five dosings were given at 24-hour intervals; (2) weekly matings were for seven rather than eight weeks; and (3) the same control (positive and vehicle) groups used for the acute dosing also served as reference groups for the subacute assay.

Necropsy

One-fourth of the pregnant females in each group were sacrificed on each of four days starting on the 15th day after the first day of breeding. This schedule allowed for sacrifice of females between 11 and 18 days of pregnancy. A complete autopsy of each female was done to determine if there was intercurrent infection, since such a condition can induce pre-implantation loss and early fetal deaths (Rhorboin, Humangenetik 6, 345, 1968).

Observations

At time of sacrifice, each female was scored for early fetal deaths, late fetal deaths, living fetuses (all of which provide a total implant score), corpora lutea, and pre-implantation loss (determined by subtracting the total implant score from the total corpora lutea score).

Evaluation

The following parameters indicate an effect in dominant lethal gene studies: total implants (live fetuses plus early and late fetal deaths), total dead (early and late fetal deaths), dead implants per total implants, and pre-implantation loss (calculated as the difference between the total corpora lutea and total implant counts). Also evaluated were total corpora lutea because a significant increase of this parameter could influence the significance of the pre-implantation loss. Total implants, total dead, total corpora lutea, and pre-implantation loss parameters were analyzed for significance by the t-test.

The index of dead implants per total implants was analyzed statistically by the t-test on arcsine (or angular) transformed data, as described in "Experimental Design (Theory and Application)," by Walter T. Federer, The Macmillan Company, 1955. This index was computed for each female. Some differences of opinion exist regarding the appropriateness of this parameter as a measure of effect, especially the statistical evaluation.

The assumptions underlying the analysis of variance and the usual tests of significance are discussed by Eisenhart (*Biometrics* 3, 1-21, 1947); in the same issue, Cochran (*Biometrics* 3, 22-38, 1947) discusses the consequences when the assumptions underlying the analysis of variance are not fulfilled. These two papers, plus the one by Bartlett (*The use of transformations*, *Biometrics* 3, 39-52 and 9, 1947), provide background information on this subject.

All calculations were run on a Wang 700/701 System (programmable calculator).

Results and Discussion

Table 7 presents the single and multiple dose LD₅₀ values for FDA Red No. 2 (FDA No. 71-23). Ten g/kg of bodyweight of compound 71-23 given orally as a suspension in corn oil caused no deaths nor adverse effects, except transient depression of the animals for a few hours following dosing. Multiple doses of 5 g/kg caused no observable effects. Thus, the maximum dose of compound No. 71-23 used in these mutagenic assays was 5 g/kg of bodyweight.

DOMINANT LETHAL GENE-RAT

Table 7

ORAL LD₅₀

Compound: FD&C Red #2
FDA No: 71-23

Single Dose ^a	> 10g/kg
Multiple Dose ^b	> 5 g/kg

^a Ten male, Sprague-Dawley rats, weighing 200-250 grams each, were fasted overnight and then administered orally specified amounts of the candidate compound dissolved or suspended in corn oil.

^b Ten male, nonfasted Sprague-Dawley rats, weighing 200-250 g each, were administered orally specified amounts of the candidate compound dissolved or suspended in corn oil.

In Table 8, summary data of the average implantations per pregnant female showed no consistent effect of Red No. 2 on this parameter, while the TEM-treated group had a marked drop in implants during the second, third, and fourth weeks. First-week data of number of implants for all groups of the multiple-treated Red No. 2 rats were significantly less than the controls, but had implant data comparable to the controls throughout the remaining seven weeks of the experiment. Taking into account the time required for sperm development and maturation, these first-week slight effects appear to be more closely related to the stress of heavy dosing, rather than to a metabolic response.

Dead implants per pregnant female are summarized in Table 9. Again, the TEM group was markedly affected through the sixth week. Data for compound 71-23 rats showed no statistical differences from the controls throughout the first seven weeks. In the last week, the mid-dose rats (2.5 g/kg) showed a significant increase in dead implants per female for the group where the male was singly treated with Red No. 2, while the opposite effect, a significant decrease in dead implant occurrence, was seen in the multiple-dosed group.

The same types of scattered responses were obtained in statistical treatment of: dead implants per total implants (Table 10), corpora lutea per pregnant female (Table 11), and pre-implantation loss per pregnant female (Table 12).

Careful review, study and statistical treatment of the data fail to show that FD&C Red No. 2 (FDA compound No. 71-23) is a mutagen in the rat by this dominant lethal gene test.

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DOMINANT LETHAL GENE-RAT

Table 8

AVERAGE IMPLANTATIONS PER PREGNANT FEMALE

Compound: FD&C Red #2
FDA No: 71-23

Week of study	Control (10 ml/kg)	TEM (0.2 mg/kg)	71-23 (30 mg/kg)	71-23 (2.5 g/kg)	71-23 (5 g/kg)
<u>Acute-Single Dose</u>					
1	202/16=12.6	183/15=12.2	194/16=12.1	250/19=13.2	216/18=12.0
2	199/16=12.4	124/15= 8.3**	150/12=12.5	169/16=10.6*	201/18=11.2
3	224/17=13.2	179/18= 9.9**	168/14=12.0	200/16=12.5	174/15=11.6**
4	228/18=12.7	74/15= 4.9**	203/17=11.9	228/18=12.7	172/16=10.8
5	233/20=11.7	186/17=10.9	217/16=13.6**I	230/20=11.5	226/19=11.9
6	234/20=11.7	164/15=10.9	133/13=10.2	190/17=11.2	233/20=11.7
7	223/20=11.2	175/16=10.9	171/15=11.4	211/18=11.7	208/20=10.4
8	228/20=11.4	185/17=10.9	172/15=11.5	221/20=11.1	220/20=11.0
<u>Subacute-Multiple Dose</u>					
1	210/16=13.1		124/12=10.3**	134/12=11.2*	170/17=10.0**
2	221/17=13.0		235/18=13.1	247/18=13.7	230/17=13.5
3	217/17=12.8		215/17=12.6	216/17=12.7	188/16=11.8
4	222/17=13.1		245/20=12.3	239/19=12.6	234/20=11.7
5	246/20=12.3		227/19=11.9	228/20=11.4	246/20=12.3
6	230/20=11.5		227/19=11.9	239/20=12.0	236/20=11.8
7	215/18=11.9		221/20=11.1	243/20=12.2	229/20=11.5

* Significant at P <0.05.

** Significant at P <0.01.

I = Increased over control.

DOMINANT LETHAL GENE-RAT

Table 9

AVERAGE DEAD IMPLANTS PER PREGNANT FEMALE

Compound: FD&C Red #2
FDA No: 71-23

Week of study	Control (10 ml/kg)	TEM (0.2 mg/kg)	71-23 (30 mg/kg)	71-23 (2.5 g/kg)	71-23 (5 g/kg)
<u>Acute-Single Dose</u>					
1	15/16=0.94	70/15=4.67**	8/16=0.50	29/19=1.53	9/18=0.50
2	9/16=0.56	103/15=6.87**	12/12=1.00	6/16=0.38	15/18=0.83
3	11/17=0.65	138/18=7.67**	12/14=0.86	7/16=0.44	7/15=0.47
4	16/18=0.89	68/15=4.53**	21/17=1.24	22/18=1.22	16/16=1.00
5	12/20=0.60	70/17=4.12**	16/16=1.00	13/20=0.65	11/19=0.58
6	6/20=0.30	25/15=1.67**	11/13=0.85	15/17=0.88	6/20=0.30
7	10/20=0.50	12/16=0.75	13/15=0.87	13/18=0.72	14/20=0.70
8	10/20=0.50	15/17=0.88	15/15=1.00	23/20=1.15*	16/20=0.80
<u>Subacute-Multiple Dose</u>					
1	18/16=1.13		9/12=0.75	12/12=1.00	13/17=0.76
2	15/17=0.88		10/18=0.56	28/18=1.56	16/17=0.94
3	23/17=1.35		9/17=0.53	15/17=0.88	10/16=0.63
4	9/17=0.53		13/20=0.65	15/19=0.79	12/20=0.60
5	11/20=0.55		6/19=0.32	15/20=0.75	22/20=1.10
6	14/20=0.70		17/19=0.89	27/20=1.35	9/20=0.45
7	25/18=1.39		15/20=0.75	6/20=0.30**D	17/20=0.85

* Significant at P <0.05.

** Significant at P <0.01.

D=Decrease below control.

Table 10
DEAD IMPLANTS/TOTAL IMPLANTS

Compound: FD&C Red #2
FDA No: 71-23

Week of study	Control (10 ml/kg)	TEM (0.2 mg/kg)	71-23 (30 mg/kg)	71-23 (2.5 g/kg)	71-23 (5 g/kg)
Acute-Single Dose					
1	15/202=0.07	70/183=0.38**	8/194=0.04	29/250=0.12	9/216=0.04
2	9/199=0.05	103/124=0.83**	12/150=0.08	6/169=0.04	15/201=0.07
3	11/224=0.05	138/179=0.77**	12/168=0.07	7/200=0.04	7/174=0.04
4	16/228=0.07	68/74=0.92**	21/203=0.10	22/228=0.10	16/172=0.09
5	12/233=0.05	70/186=0.38**	16/217=0.07	12/230=0.06	11/226=0.05
6	6/234=0.03	25/164=0.15**	11/133=0.08	15/190=0.08	6/233=0.03
7	10/223=0.04	12/175=0.07	13/171=0.08	13/211=0.06	14/208=0.07
8	10/228=0.04	15/185=0.08	15/172=0.09	23/221=0.10*	16/220=0.07
Subacute-Multiple					
1	18/210=0.09		9/124=0.07	12/134=0.09	13/170=0.08
2	15/221=0.07		10/235=0.04	28/247=0.11	16/230=0.07
3	23/217=0.11		9/215=0.04	15/216=0.07	10/188=0.05
4	9/222=0.04		13/245=0.05	15/239=0.06	12/234=0.05
5	11/246=0.04		6/227=0.03	15/228=0.07	22/246=0.09
6	14/230=0.06		17/227=0.07	27/239=0.11	9/236=0.04
7	25/215=0.12		15/221=0.07	6/243=0.02	17/229=0.07

* Significant at P <0.05

** Significant at P <0.01.

Table 11

AVERAGE CORPORA LUTEA PER PREGNANT FEMALE

Compound: FD&C Red #2
 FDA No: 71-23

Week of study	Control (10 ml/kg)	TEM (0.2 mg/kg)	71-23 (30 mg/kg)	71-23 (2.5 g/kg)	71-23 (5 g/kg)
<u>Acute-Single Dose</u>					
1	222/16=13.9	229/15=15.3	223/16=13.9	268/19=14.1	241/18=13.4
2	213/16=13.3	188/15=12.5	167/12=13.9	209/16=13.1	234/18=13.0
3	236/17=13.9	279/18=15.5	199/14=14.2	226/16=14.1	185/15=12.3**
4	246/18=13.7	192/15=12.8	237/17=13.9	243/18=13.5	217/16=13.6
5	256/20=12.8	241/17=14.2**I	234/16=14.6**I	245/20=12.3	256/19=13.5
6	245/20=12.3	189/15=12.6	165/13=12.7	211/17=12.4	256/20=12.8
7	249/20=12.5	204/16=12.8	194/15=12.9	233/18=12.9	248/20=12.4
8	239/20=12.0	205/17=12.1	194/15=12.9*I	257/20=12.9	239/20=12.0
<u>Subacute-Multiple Dose</u>					
1	222/16=13.9		148/12=12.3**	150/12=12.5**	212/17=12.5**
2	230/17=13.5		258/18=14.3	264/18=14.7*I	254/17=14.9*I
3	241/17=14.2		244/17=14.4	239/17=14.1	208/16=13.0
4	237/17=13.9		273/20=13.7	275/19=14.5	256/20=12.8
5	262/20=13.1		255/19=13.4	246/20=12.3	257/20=12.9
6	268/20=13.4		265/19=14.0	277/20=13.9	268/20=13.4
7	220/18=12.2		243/20=12.2	248/20=12.4	254/20=12.7

* Significant at P <0.05.

** Significant at P <0.01.

I = Increase over control.

Table 12

AVERAGE PREIMPLANTATION LOSS PER PREGNANT FEMALE

Compound: FD&C Red #2
 FDA No: 71-23

Week of study	Control (10 ml/kg)	TEM (0.2 mg/kg)	71-23 (30 mg/kg)	71-23 (2.5 g/kg)	71-23 (5 g/kg)
<u>Acute-Single Dose</u>					
1	20/16=1.25	46/15=3.07	29/16=1.81	18/19=0.95	25/18=1.39
2	14/16=0.88	64/15=4.27**	17/12=1.42	40/16=2.50	33/18=1.83
3	12/17=0.71	100/18=5.56*	31/14=2.21	26/16=1.63	11/15=0.73
4	18/18=1.00	118/15=7.87**	34/17=2.00	15/18=0.83	45/16=2.81*
5	23/20=1.15	55/17=3.24*	17/16=1.06	15/20=0.75	30/19=1.58
6	11/20=0.55	25/15=1.67	32/13=2.46	21/17=1.24	23/20=1.15
7	26/20=1.30	29/16=1.81	23/15=1.53	22/18=1.22	40/20=2.00
8	11/20=0.55	20/17=1.18	22/15=1.47	36/20=1.80*	19/20=0.95
<u>Subacute-Multiple Dose</u>					
1	12/16=0.75		24/12=2.00	16/12=1.33	42/17=2.47*
2	9/17=0.53		23/18=1.28	17/18=0.94	24/17=1.35
3	24/17=1.41		29/17=1.71	23/17=1.35	20/16=1.25
4	15/17=0.88		28/20=1.40	36/19=1.89	22/20=1.10
5	16/20=0.80		28/19=1.47	18/20=0.90	11/20=0.55
6	38/20=1.90		38/19=2.00	38/20=1.90	32/20=1.60
7	5/18=0.28		22/20=1.10	5/20=0.25	25/20=1.25

* Significant at P <0.05

** Significant at P <0.01



STANFORD RESEARCH INSTITUTE
SRI INTERNATIONAL

June 1972

Compound Report No. 1 (Addendum)

STUDY OF MUTAGENIC EFFECTS OF FD&C Red No. 2 (FDA No. 71-23)

Prepared for:

DHEW/PUBLIC HEALTH SERVICE
Food and Drug Administration
Rockville, Maryland

Contract No. FDA 71-267

SRI Project LSU-1348

Submitted by:

G. W. Newell and W. A. Maxwell

Approved:

**W. A. Skinner, Executive Director
Life Sciences Division**

INTRODUCTION

Under contract to the Food and Drug Administration, Stanford Research Institute is examining the mutagenicity of 14 selected chemical compounds (Contract No. FDA 71-267). This report is an addendum to the first compound report of tests conducted on FD&C Red No. 2 (FDA No. 71-23).

The initial results reported in Compound Report No. 1 in January 1972 were somewhat spurious for the host-mediated assay. These tests were repeated and are reported here.

SUMMARY

FD&C Red No. 2 (71-23) did not produce any measurable mutagenic response in two strains of Salmonella typhimurium or alteration in the mitotic recombination frequency for Saccharomyces cerevisiae.

RESULTS AND DISCUSSION

The methodologies used in these tests are described in Compound Report No. 1, January 1972.

Table 1 presents a summary of the host-mediated assay results for FD&C Red No. 2(71-23). Table 2 contains the data obtained on each individual mouse. This table is a computer printout of the calculations made on the data obtained for each mouse. Because of the nature of the computer, it is necessary to exceed its maximum number of significant figures to obtain a value as an exponent. For this reason, 12 figures are printed out. However, only three significant figures are used for calculations and reporting the results as summarized in Table 1.

As can be seen in Table 1, no mutagenic response was observed for the two Salmonella typhimurium strains tested when mice were treated with the test compound. The mitotic recombination frequency of Saccharomyces cerevisiae was not affected.

The slightly increased mutation frequencies obtained in the acute studies with TA-1530 are small compared with the positive control. The extremely low value for the negative control indicates that it may be somewhat low, particularly when compared to the spontaneous mutation

frequencies normally obtained for TA-1530. The MF_t/MF_c for the positive control is approximately three to four times that normally used. If the negative control was adjusted by this value, the treated groups would be essentially the same as the negative control.

In studies subsequent to the initial report in January 1972 on FD&C Red No. 2, it was determined that high levels of contamination were present in the stock cultures of bacteria and were responsible for the spurious results. Apparently this contamination was present in the original inoculum. Attempts to purify the stock cultures were not successful, and, therefore, a new culture was obtained from Dr. Bruce Ames, University of California, Berkeley. All tests using these new cultures have been consistent and reproducible.

Table 1
HOST MEDIATED ASSAY
SUMMARY OF DATA

Compound No.: 71-23 (FD&C Red #2)

A. Acute

Treatment	Organism					
	Salmonella				Saccharomyces	
	G <small>46</small>		TA 1530		D-3	
	MF	MFT/ MFC	MF	MFT/ MFC	RF	RFT/ RFC
Maximum	4.09×10^{-9}	0.62	3.59×10^{-8}	13.25 4.87	9.49×10^{-5}	1.23
Intermediate	3.26×10^{-9}	0.49	2.50×10^{-8}	9.22	2.14×10^{-4}	2.78
Low Level	4.14×10^{-9}	0.62	2.46×10^{-8}	9.07	9.03×10^{-5}	1.17
Control (+)	2.00×10^{-7}	30.01	3.94×10^{-7}	145.38	9.98×10^{-4}	12.93
Control (-)	6.65×10^{-9}	1.00	2.71×10^{-8}	1.00	7.72×10^{-5}	1.00

B. Subacute

Treatment	Organism					
	Salmonella				Saccharomyces	
	G <small>46</small>		TA 1530		D-3	
	MF	MFT/ MFC	MF	MFT/ MFC	RF	RFT/ RFC
Maximum	2.73×10^{-9}	1.31	1.65×10^{-8}	0.42	1.53×10^{-4}	0.92
Intermediate	2.43×10^{-9}	1.17	7.17×10^{-8}	1.83	1.51×10^{-4}	0.90
Low Level	1.52×10^{-9}	0.73	6.87×10^{-8}	1.75	1.39×10^{-4}	0.83
Control (-)	2.07×10^{-9}	1.00	3.91×10^{-8}	1.00	1.67×10^{-4}	1.00

Table 2

HOST MEDIATED ASSAY
INDIVIDUAL MOUSE DATA

Compound No.: 71-23 (F&C Red #2)

Organism: G-46

Treatment: (+) CONTROL

A. Acute

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>
1	.55833333330ex 02	.985000000000ex 09	.566835871401ex-07
2	.26833333333ex 03	.616666666665ex 09	.435135135135ex-06
3	.45000000000ex 02	.102666666666ex 10	.438311688314ex-07
4	.31666666666ex 03	.98333333330ex 09	.322033898305ex-06
5	.635000000000ex 03	.111000000000ex 10	.572072072072ex-06
6	.641666666665ex 02	.168333333333ex 10	.381188118811ex-07
7	.316666666666ex 02	.955000000000ex 09	.331588132634ex-07
8	.40833333333ex 02	.110833333333ex 10	.368421052632ex-07
9	.321000000000ex 03	.122833333333ex 10	.261329715061ex-06
.199911700771ex-06			

B. Subacute

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>

Table 2 (continued)

HOST MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red #2)Organism: G-46Treatment: (-) CONTROL

A. Acute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.33333333333ex 01	.91166666666ex 09	.365630712980ex-08
2	.14166666666ex 02	.67833333330ex 09	.208845208845ex-07
3	.58333333330ex 01	.84000000000ex 09	.694444444440ex-08
4	.83333333330ex 01	.95166666665ex 09	.875656742554ex-08
5	.10000000000ex 01	.62833333330ex 09	.159151193634ex-08
6	.66666666666ex 01	.10333333333ex 10	.645161290323ex-08
7	.25000000000ex 01	.82833333330ex 09	.301810865192ex-08
8	.16666666666ex 01	.85666666666ex 09	.194552529182ex-08
			.665607483342ex-08

B. Subacute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.83333333330ex 00	.18833333333ex 10	.442477876105ex-09
2	.50000000000ex 01	.18333333333ex 10	.272727272727ex-08
3	.41666666666ex 01	.20666666666ex 10	.201612903226ex-08
4	.41666666666ex 01	.13333333333ex 10	.31250000000ex-08
5	.16666666666ex 01	.84166666665ex 09	.198019801979ex-08
6	.25000000000ex 01	.11733333333ex 10	.213068181818ex-08
			.207029324558ex-08

Table 2 (continued)

HOST MEDIATED ASSAY
INDIVIDUAL MOUSE DATA

Compound No.: 71-23 (FD&C Red #2)

Organism: G-46

Treatment: MAXIMUM

A. Acute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recom- bination Frequency
	Colonies or Recom- binants/ml		
1	.16666666666ex 01	.86500000000ex 09	.192678227359ex-08
2	.50000000000ex 01	.16388888888ex 10	.305084745764ex-08
3	.83333333330ex 00	.10166666666ex 10	.819672131149ex-09
4	.83333333330ex 00	.65833333330ex 09	.126582278481ex-08
5	.16666666666ex 01	.10150000000ex 10	.164203612478ex-08
6	.16666666666ex 01	.44666666666ex 09	.373134328357ex-08
7	.66666666666ex 01	.94833333330ex 09	.702987697716ex-08
8	.33333333333ex 01	.82666666665ex 09	.403225806452ex-08
9	.10000000000ex 01	.75000000000ex 08	.13333333333ex-07
			.409244138115ex-08

B. Subacute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recom- bination Frequency
	Colonies or Recom- binants/ml		
1	.75000000000ex 01	.15666666666ex 10	.478723404257ex-08
2	.66666666666ex 01	.15166666666ex 10	.439560439561ex-08
3	.25000000000ex 01	.31500000000ex 10	.793650793650ex-09
4	.58333333330ex 01	.10466666666ex 10	.557324840764ex-08
5	.16666666666ex 01	.20500000000ex 10	.813008130078ex-09
6	.83333333330ex 00	.76500000000ex 09	.108932461873ex-08
7	.25000000000ex 01	.14666666666ex 10	.1704545455ex-08
			.273665940608ex-08

Table 2 (continued)

HOST MEDIATED ASSAY
INDIVIDUAL MOUSE DATA

Compound No.: 71-23 (F1&C Red #2)

Organism: G-46

Treatment: INTERMEDIATE

A. Acute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.833333333330ex 00	.620000000000ex 09	.134408602150ex-08
2	.166666666666ex 01	.163333333333ex 10	.102040816326ex-08
3	.250000000000ex 01	.61666666665ex 09	.405405405406ex-08
4	.166666666666ex 01	.53833333330ex 09	.309597523220ex-08
5	.166666666666ex 01	.66333333330ex 09	.251256281407ex-08
6	.166666666666ex 01	.238333333333ex 09	.699300699298ex-08
7	.500000000000ex 01	.130833333333ex 10	.382165605096ex-08
			.326310704697ex-08

B. Subacute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.166666666666ex 01	.72833333330ex 09	.228832951945ex-08
2	.250000000000ex 01	.88333333330ex 09	.283018867925ex-08
3	.250000000000ex 01	.560000000000ex 09	.446428571428ex-08
4	.83333333330ex 00	.63166666665ex 09	.131926121371ex-08
5	.33333333333ex 01	.85666666665ex 09	.389105058366ex-08
6	.166666666666ex 01	.141166666666ex 10	.118063754427ex-08
7	.166666666666ex 01	.301666666666ex 10	.552486187844ex-09
8	.500000000000ex 01	.171666666666ex 10	.291262135923ex-08
			.242985760017ex-08

Table 2 (continued)

HOST MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red #2)Organism: G-46Treatment: Low

A. Acute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.166666666666ex 01	.963333333330ex 09	.173010380622ex-08
2	.833333333330ex 00	.71166666665ex 09	.117096018735ex-08
3	.833333333330ex 00	.118333333333ex 10	.704225352111ex-09
4	.166666666666ex 01	.878333333330ex 09	.189753320683ex-08
5	.500000000000ex 01	.648333333330ex 09	.771208226225ex-08
6	.833333333330ex 00	.445000000000ex 09	.187265917602ex-08
7	.166666666666ex 01	.776666666665ex 09	.214592274677ex-08
8	.166666666666ex 02	.905000000000ex 09	.184162062614ex-07
9	.166666666666ex 01	.101833333333ex 10	.163666121112ex-08
			.414292824554ex-08

B. Subacute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.33333333333ex 01	.215000000000ex 10	.155038759689ex-08
2	.33333333333ex 01	.43666666666ex 10	.763358778626ex-09
3	.33333333333ex 01	.148333333333ex 10	.224719101123ex-08
4	.250000000000ex 01	.151666666666ex 10	.164835164835ex-08
5	.250000000000ex 01	.180000000000ex 10	.138888888888ex-08
			.151963558479ex-08

Table 2 (continued)

HOST MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red #2)Organism: TA-1530Treatment: (+) CONTROL

A. Acute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.91000000000ex 02	.71166666666ex 09	.127868852459ex-06
2	.23416666666ex 03	.79666666666ex 09	.293933054393ex-06
3	.30750000000ex 03	.58166666666ex 09	.528653295130ex-06
4	.31833333333ex 03	.94333333330ex 09	.337455830389ex-06
5	.21833333333ex 03	.66833333330ex 09	.326683291771ex-06
6	.58600000000ex 03	.64166666665ex 09	.913246753249ex-06
7	.20625000000ex 03	.48833333333ex 09	.422354948805ex-06
8	.15600000000ex 03	.77833333330ex 09	.200428265525ex-06
.393828036462ex-06			

B. Subacute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		

Table 2 (continued)

HOST MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red #2)Organism: TA-1530Treatment: (-) CONTROL

A. Acute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.33333333333ex 01	.130166666666ex 10	.256081946223ex-08
2	.166666666666ex 01	.211666666666ex 10	.787401574802ex-09
3	.83333333330ex 00	.245000000000ex 10	.340136054420ex-09
4	.83333333330ex 00	.151666666666ex 10	.549450549450ex-09
5	.150000000000ex 02	.241666666666ex 10	.620689655174ex-08
6	.83333333330ex 00	.127500000000ex 10	.653594771239ex-09
7	.116666666666ex 02	.112500000000ex 10	.103703703703ex-07
8	.250000000000ex 01	.175000000000ex 10	.142857142857ex-08
9	.250000000000ex 01	.168333333333ex 10	.148514851485ex-08
			.270915436415ex-08

B. Subacute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.20833333333ex 02	.115000000000ex 10	.181159420289ex-07
2	.300000000000ex 02	.810000000000ex 09	.370370370370ex-07
3	.916666666665ex 01	.500000000000ex 09	.183333333333ex-07
4	.40833333333ex 02	.638333333330ex 09	.639686684075ex-07
5	.216666666666ex 02	.121166666666ex 10	.178817056396ex-07
6	.300000000000ex 02	.598333333330ex 09	.501392757662ex-07
7	.300000000000ex 02	.508333333330ex 09	.590163934430ex-07
8	.416666666666ex 02	.863333333330ex 09	.482625482626ex-07
			.390943629895ex-07

Table 2 (continued)

HOST MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red #2)Organism: TA-1530Treatment: MAXIMUM

A. Acute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency	$\times 10^{-8}$
	Colonies or Recombinants/ml			
1	.40000000000ex 01	.22666666666ex 09	.176470588235ex-07	1.76
2	.916666666665ex 01	.45833333333ex 09	.19999999999ex-07	2.00
3	.916666666665ex 01	.94833333330ex 09	.966608084360ex-08	0.97
4	.18333333333ex 02	.32500000000ex 09	.56410256410lex-07	5.64
5	.10833333333ex 02	.93333333330ex 08	.116071428571ex-06	11.60
6	.10833333333ex 02	.11016666666ex 10	.983358547657ex-08	0.98
7	.14166666666ex 02	.45833333333ex 09	.309090909089ex-07	3.09
8	.15833333333ex 02	.59500000000ex 09	.266106442576ex-07	2.66
			.35893518161lex-07	

B. Subacute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.75000000000ex 01	.21333333333ex 09	.351562500000ex-07
2	.40000000000ex 01	.23333333333ex 09	.171428571428ex-07
3	.50000000000ex 01	.35333333333ex 09	.141509433962ex-07
4	.83333333330ex 01	.83333333330ex 09	.100000000000ex-07
5	.16666666666ex 01	.27500000000ex 09	.6060606058ex-08
			.165021313199ex-07

Table 2(continued)

HOST MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red #2)Organism: TA-1530Treatment: INTERMEDIATE

A. Acute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.170000000000ex 02	.731666666665ex 09	.232346241458ex-07
2	.291666666666ex 02	.956666666665ex 09	.304878048780ex-07
3	.290000000000ex 02	.107333333333ex 10	.270186335404ex-07
4	.191666666666ex 02	.136166666666ex 10	.140758873929ex-07
5	.125000000000ex 02	.365000000000ex 09	.342465753424ex-07
6	.166666666666ex 02	.360000000000ex 09	.462962962961ex-07
7	.133333333333ex 02	.871666666665ex 09	.152963671128ex-07
8	.100000000000ex 02	.109500000000ex 10	.913242009132ex-08
			.249735760997ex-07

B. Subacute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.400000000000ex 02	.361666666666ex 09	.110599078341ex-06
2	.466666666666ex 02	.596666666665ex 09	.782122905029ex-07
3	.466666666666ex 02	.873333333330ex 09	.534351145039ex-07
4	.283333333333ex 02	.253333333333ex 09	.111842105263ex-06
5	.275000000000ex 02	.305000000000ex 09	.901639344262ex-07
6	.608333333330ex 02	.848333333330ex 09	.717092337916ex-07
7	.325000000000ex 02	.536666666665ex 09	.605590062113ex-07
8	.300000000000ex 02	.825000000000ex 09	.363636363636ex-07
9	.266666666666ex 02	.818333333330ex 09	.325865580448ex-07
			.717189952715ex-07

Table 2 (continued)

HOST MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red #2)Organism: TA-1530Treatment: Low

A. Acute

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>
1	.250000000000ex 02	.158333333333ex 10	.157894736842ex-07
2	.141666666666ex 02	.81833333330ex 09	.173116089612ex-07
3	.58333333330ex 01	.160000000000ex 10	.364583333331ex-08
4	.133333333333ex 02	.841666666665ex 09	.158415841584ex-07
5	.266666666666ex 02	.898333333330ex 09	.296846011132ex-07
6	.133333333333ex 02	.243333333333ex 09	.547945205478ex-07
7	.250000000000ex 02	.705000000000ex 09	.354609929078ex-07
			.246469449578ex-07

B. Subacute

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>
1	.225000000000ex 02	.515000000000ex 09	.436893203883ex-07
2	.370000000000ex 02	.356666666666ex 09	.103738317757ex-06
3	.275000000000ex 02	.431666666666ex 09	.637065637066ex-07
4	.350000000000ex 02	.520000000000ex 09	.673076923076ex-07
5	.400000000000ex 02	.595000000000ex 09	.672268907563ex-07
6	.358333333333ex 02	.731666666665ex 09	.489749430524ex-07
7	.491666666666ex 02	.568333333330ex 09	.865102639300ex-07
			.687362845565ex-07

Table 2 (continued)

HOST MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red #2)Organism: D-3Treatment: (+) CONTROL

A. Acute

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>
1	.290000000000ex 05	.140000000000ex 09	.207142857142ex-03
2	.270000000000ex 05	.281666666666ex 08	.958579881659ex-03
3	.285000000000ex 05	.253333333333ex 08	.112500000000ex-02
4	.300000000000ex 05	.621666666665ex 08	.482573726542ex-03
5	.250000000000ex 05	.150000000000ex 08	.166666666666ex-02
6	.230000000000ex 05	.160000000000ex 08	.143750000000ex-02
7	.220000000000ex 05	.198333333333ex 08	.110924369748ex-02
.998100975640ex-03			

B. Subacute

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>

Table 2 (continued)

HOST MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red #2)Organism: D-3Treatment: (-) CONTROL

A. Acute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.150000000000ex 04	.470000000000ex 08	.319148936170ex-04
2	.200000000000ex 04	.223333333333ex 08	.895522388061ex-04
3	.300000000000ex 04	.516666666665ex 08	.580645161292ex-04
4	.100000000000ex 04	.543333333330ex 08	.184049079755ex-04
5	.450000000000ex 04	.558333333330ex 08	.805970149258ex-04
6	.200000000000ex 04	.293333333333ex 08	.681818181818ex-04
7	.200000000000ex 04	.333333333333ex 08	.600000000000ex-04
8	.450000000000ex 04	.285000000000ex 08	.157894736842ex-03
9	.300000000000ex 04	.485000000000ex 08	.618556701030ex-04
10	.300000000000ex 04	.206666666666ex 08	.145161290323ex-03
			.771627086901ex-04

B. Subacute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.250000000000ex 04	.226666666666ex 08	.110294117647ex-03
2	.450000000000ex 04	.191666666666ex 08	.234782608696ex-03
3	.250000000000ex 04	.246666666666ex 08	.101351351351ex-03
4	.100000000000ex 04	.755000000000ex 07	.132450331125ex-03
5	.150000000000ex 04	.178333333333ex 08	.841121495328ex-04
6	.200000000000ex 04	.146666666666ex 08	.136363636364ex-03
7	.300000000000ex 04	.200000000000ex 08	.150000000000ex-03
8	.500000000000ex 04	.326666666666ex 08	.153061224490ex-03
9	.450000000000ex 04	.190000000000ex 08	.236842105263ex-03
10	.300000000000ex 04	.917500000000ex 07	.326975476839ex-03
			.166623300129ex-03

Table 2(continued)

HOST MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red #2)Organism: D-3Treatment: MAXIMUM

A. Acute

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>
1	.400000000000ex 04	.301666666666ex 08	.132596685083ex-03
2	.100000000000ex 04	.383333333333ex 08	.260869565217ex-04
3	.300000000000ex 04	.260000000000ex 08	.115384615384ex-03
4	.250000000000ex 04	.265000000000ex 08	.943396226415ex-04
5	.450000000000ex 04	.446666666666ex 08	.100746268656ex-03
6	.600000000000ex 04	.441666666666ex 08	.135849056603ex-03
7	.250000000000ex 04	.420000000000ex 08	.595238095238ex-04
			.949324306301ex-04

B. Subacute

<u>Mouse No.</u>	<u>Ave. No. Mutant Colonies or Recombinants/ml</u>	<u>Ave. No. Colony Forming Units/ml</u>	<u>Mutation or Recombination Frequency</u>
1	.500000000000ex 04	.170000000000ex 08	.294117647058ex-03
2	.200000000000ex 04	.248333333333ex 08	.805369127517ex-04
3	.450000000000ex 04	.473333333333ex 08	.950704225352ex-04
4	.300000000000ex 04	.144750000000ex 08	.207253886010ex-03
5	.350000000000ex 04	.386666666666ex 08	.905172413794ex-04
6	.200000000000ex 04	.173333333333ex 08	.115384615384ex-03
7	.350000000000ex 04	.160000000000ex 08	.218750000000ex-03
8	.350000000000ex 04	.290000000000ex 08	.120689655172ex-03
			.152790047535ex-03

Table 2 (continued)

HOST MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red #2)Organism: D-3Treatment: INTERMEDIATE

A. Acute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.600000000000ex 04	.701666666665ex 08	.855106888363ex-04
2	.650000000000ex 04	.335000000000ex 08	.194029850746ex-03
3	.350000000000ex 04	.233333333333ex 08	.150000000000ex-03
4	.150000000000ex 04	.283333333333ex 07	.529411764706ex-03
5	.44444444444ex 04	.136666666666ex 08	.325203252033ex-03
6	.600000000000ex 04	.333333333333ex 08	.180000000000ex-03
7	.400000000000ex 04	.198333333333ex 08	.201680672269ex-03
8	.100000000000ex 04	.516666666665ex 07	.193548387097ex-03
9	.227272727272ex 04	.350000000000ex 08	.649350649348ex-04
			.213813297844ex-03

B. Subacute

Mouse No.	Ave. No. Mutant Colonies or Recombinants/ml	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
1	.750000000000ex 04	.435000000000ex 08	.172413793103ex-03
2	.300000000000ex 04	.258333333333ex 08	.116129032258ex-03
3	.350000000000ex 04	.306666666666ex 08	.114130434782ex-03
4	.300000000000ex 04	.134500000000ex 08	.223048327137ex-03
5	.200000000000ex 04	.195000000000ex 08	.102564102564ex-03
6	.200000000000ex 04	.156666666666ex 08	.127659574468ex-03
7	.300000000000ex 04	.138333333333ex 08	.216867469880ex-03
8	.250000000000ex 04	.133000000000ex 08	.187969924812ex-03
9	.350000000000ex 04	.166000000000ex 08	.210843373493ex-03
10	.100000000000ex 04	.276666666666ex 08	.361445783133ex-04
			.150777061080ex-03

Table 2 (concluded)

HOST MEDIATED ASSAY
INDIVIDUAL MOUSE DATACompound No.: 71-23 (FD&C Red #2)Organism: D-3Treatment: Low

A. Acute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.250000000000ex 04	.360000000000ex 08	.694444444444ex-04
2	.400000000000ex 04	.448333333333ex 08	.892193308550ex-04
3	.200000000000ex 04	.506666666665ex 08	.394736842106ex-04
4	.150000000000ex 04	.396666666666ex 08	.378151260504ex-04
5	.300000000000ex 04	.423333333333ex 08	.708661417323ex-04
6	.850000000000ex 04	.485000000000ex 08	.175257731958ex-03
7	.300000000000ex 04	.350000000000ex 08	.857142857142ex-04
8	.450000000000ex 04	.383333333333ex 08	.117391304347ex-03
9	.100000000000ex 04	.783333333330ex 07	.127659574468ex-03
			.903157359753ex-04

B. Subacute

Mouse No.	Ave. No. Mutant	Ave. No. Colony Forming Units/ml	Mutation or Recombination Frequency
	Colonies or Recombinants/ml		
1	.350000000000ex 04	.173333333333ex 08	.201923076923ex-03
2	.600000000000ex 04	.381666666666ex 08	.157205240174ex-03
3	.350000000000ex 04	.251666666666ex 08	.139072847682ex-03
4	.350000000000ex 04	.593333333330ex 08	.589887640452ex-04
5	.300000000000ex 04	.228333333333ex 08	.131386861314ex-03
6	.350000000000ex 04	.218333333333ex 08	.160305343511ex-03
7	.250000000000ex 04	.148333333333ex 08	.168539325843ex-03
8	.400000000000ex 04	.235000000000ex 08	.170212765957ex-03
9	.100000000000ex 04	.166666666666ex 08	.600000000002ex-04
			.138626025048ex-03



STANFORD RESEARCH INSTITUTE

August 1972

Compound Report No. 1 (Statistical Addendum--Dominant Lethal Gene Data)

STUDY OF MUTAGENIC EFFECTS OF FD&C RED NO. 2 (FDA No. 71-23)

Prepared for:

DHEW/PUBLIC HEALTH SERVICE
Food and Drug Administration
Rockville, Maryland

Contract No. FDA 71-267

SRI Project LSU-1348

Submitted by:

G. W. Newell and W. A. Maxwell

Approved:

A handwritten signature in black ink that appears to read "W.A. Skinner".

W. A. Skinner, Executive Director
Life Sciences Division

STATISTICAL SUMMARY

This addendum presents statistical treatment of the dominant lethal gene data for FD&C Red No. 2, using the procedural outline of Miss Janet Springer, FDA. A description of the statistical procedures and an explanation of how the computations are accomplished were presented as Appendix A of Compound Report No. 8, Guar Gum (71-16). Summary tables of experimental data also are included for reference.

A review of these statistical evaluations continues to support the conclusions presented in the main report: i.e., FD&C Red No. 2 is not a mutagenic substance by the dominant lethal gene test.

DOMINANT LETHAL GENE-RAT

Table 8

AVERAGE IMPLANTATIONS PER PREGNANT FEMALE

Compound: FD&C Red #2
FDA No: 71-23

Week of study	Control (10 ml/kg)	TEM (0.2 mg/kg)	71-23 (30 mg/kg)	71-23 (2.5 g/kg)	71-23 (5 g/kg)
<u>Acute-Single Dose</u>					
1	202/16=12.6	183/15=12.2	194/16=12.1	250/19=13.2	216/18=12.0
2	199/16=12.4	124/15= 8.3**	150/12=12.5	169/16=10.6*	201/18=11.2
3	224/17=13.2	179/18= 9.9*	168/14=12.0	200/16=12.5	174/15=11.6*
4	228/18=12.7	74/15= 4.9**	203/17=11.9	228/18=12.7	172/16=10.8
5	233/20=11.7	186/17=10.9	217/16=13.6**I	230/20=11.5	226/19=11.9
6	234/20=11.7	164/15=10.9	133/13=10.2	190/17=11.2	233/20=11.7
7	223/20=11.2	175/16=10.9	171/15=11.4	211/18=11.7	208/20=10.4
8	228/20=11.4	185/17=10.9	172/15=11.5	221/20=11.1	220/20=11.0
<u>Subacute-Multiple Dose</u>					
1	210/16=13.1		124/12=10.3**	134/12=11.2*	170/17=10.0**
2	221/17=13.0		235/18=13.1	247/18=13.7	230/17=13.5
3	217/17=12.8		215/17=12.6	216/17=12.7	188/16=11.8
4	222/17=13.1		245/20=12.3	239/19=12.6	234/20=11.7
5	246/20=12.3		227/19=11.9	228/20=11.4	246/20=12.3
6	230/20=11.5		227/19=11.9	239/20=12.0	236/20=11.8
7	215/18=11.9		221/20=11.1	243/20=12.2	229/20=11.5

* Significant at P <0.05,

** Significant at P <0.01,

I = Increased over control.

DOMINANT LETHAL GENE-RAT

Table 9

AVERAGE DEAD IMPLANTS PER PREGNANT FEMALE

Compound: FD&C Red #2
FDA No: 71-23

Week of study	Control (10 ml/kg)	TEM (0.2 mg/kg)	71-23 (30 mg/kg)	71-23 (2.5 g/kg)	71-23 (5 g/kg)
<u>Acute-Single Dose</u>					
1	15/16=0.94	70/15=4.67**	8/16=0.50	29/19=1.53	9/18=0.50
2	9/16=0.56	103/15=6.87**	12/12=1.00	6/16=0.38	15/18=0.83
3	11/17=0.65	138/18=7.67**	12/14=0.86	7/16=0.44	7/15=0.47
4	16/18=0.89	68/15=4.53**	21/17=1.24	22/18=1.22	16/16=1.00
5	12/20=0.60	70/17=4.12**	16/16=1.00	13/20=0.65	11/19=0.58
6	6/20=0.30	25/15=1.67**	11/13=0.85	15/17=0.88	6/20=0.30
7	10/20=0.50	12/16=0.75	13/15=0.87	13/18=0.72	14/20=0.70
8	10/20=0.50	15/17=0.88	15/15=1.00	23/20=1.15	16/20=0.80
<u>Subacute-Multiple Dose</u>					
1	18/16=1.13		9/12=0.75	12/12=1.00	13/17=0.76
2	15/17=0.88		10/18=0.56	28/18=1.56	16/17=0.94
3	23/17=1.35		9/17=0.53	15/17=0.88	10/16=0.63
4	9/17=0.53		13/20=0.65	15/19=0.79	12/20=0.60
5	11/20=0.55		6/19=0.32	15/20=0.75	22/20=1.10
6	14/20=0.70		17/19=0.89	27/20=1.35	9/20=0.45
7	25/18=1.39		15/20=0.75	6/20=0.30**D	17/20=0.85

* Significant at P <0.05.

** Significant at P <0.01.

D=Decrease below control.

DOMINANT LETHAL GENE-RAT

Table 10
DEAD IMPLANTS/TOTAL IMPLANTS

Compound: FD&C Red #2
FDA No: 71-23

Week of study	Control (10 ml/kg)	TEM (0.2 mg/kg)	71-23 (30 mg/kg)	71-23 (2.5 g/kg)	71-23 (5 g/kg)
<u>Acute-Single Dose</u>					
1	15/202=0.07	70/183=0.38**	8/194=0.04	29/250=0.12	9/216=0.04
2	9/199=0.05	103/124=0.83**	12/150=0.08	6/169=0.04	15/201=0.07
3	11/224=0.05	138/179=0.77**	12/168=0.07	7/200=0.04	7/174=0.04
4	16/228=0.07	68/74=0.92**	21/203=0.10	22/228=0.10	16/172=0.09
5	12/233=0.05	70/186=0.38**	16/217=0.07	12/230=0.06	11/226=0.05
6	6/234=0.03	25/164=0.15**	11/133=0.08 *	15/190=0.08	6/233=0.03
7	10/223=0.04	12/175=0.07	13/171=0.08	13/211=0.06	14/208=0.07
8	10/228=0.04	15/185=0.08	15/172=0.09	23/221=0.10*	16/220=0.07
<u>Subacute-Multiple</u>					
1	18/210=0.09		9/124=0.07	12/134=0.09	13/170=0.08
2	15/221=0.07		10/235=0.04	28/247=0.11	16/230=0.07
3	23/217=0.11		9/215=0.04	15/216=0.07	10/188=0.05
4	9/222=0.04		13/245=0.05	15/239=0.06	12/234=0.05
5	11/246=0.04		6/227=0.03	15/228=0.07	22/246=0.09
6	14/230=0.06		17/227=0.07	27/239=0.11	9/236=0.04
7	25/215=0.12		15/221=0.07	6/243=0.02 **D	17/229=0.07

* Significant at P <0.05.

** Significant at P <0.01.

D Decrease below control

DOMINANT LETHAL GENE-RAT

Table 11

AVERAGE CORPORA LUTEA PER PREGNANT FEMALE

Compound: FD&C Red #2
FDA No: 71-23

Week of study	Control (10 ml/kg)	TEM (0.2 mg/kg)	71-23 (30 mg/kg)	71-23 (2.5 g/kg)	71-23 (5 g/kg)
<u>Acute-Single Dose</u>					
1	222/16=13.9	229/15=15.3	223/16=13.9	268/19=14.1	241/18=13.4
2	213/16=13.3	188/15=12.5	167/12=13.9	209/16=13.1	234/18=13.0
3	236/17=13.9	279/18=15.5	199/14=14.2	226/16=14.1	185/15=12.3**
4	246/18=13.7	192/15=12.8	237/17=13.9	243/18=13.5	217/16=13.6
5	256/20=12.8	241/17=14.2**I	234/16=14.6**I	245/20=12.3	256/19=13.5
6	245/20=12.3	189/15=12.6	165/13=12.7	211/17=12.4	256/20=12.8
7	249/20=12.5	204/16=12.8	194/15=12.9	233/18=12.9	248/20=12.4
8	239/20=12.0	205/17=12.1	194/15=12.9*I	257/20=12.9	239/20=12.0
<u>Subacute-Multiple Dose</u>					
1	222/16=13.9		148/12=12.3**	150/12=12.5**	212/17=12.5**
2	230/17=13.5		258/18=14.3	264/18=14.7*I	254/17=14.9*I
3	241/17=14.2		244/17=14.4	239/17=14.1	208/16=13.0
4	237/17=13.9		273/20=13.7	275/19=14.5	256/20=12.8
5	262/20=13.1		255/19=13.4	246/20=12.3	257/20=12.9
6	268/20=13.4		265/19=14.0	277/20=13.9	268/20=13.4
7	220/18=12.2		243/20=12.2	248/20=12.4	254/20=12.7

* Significant at P <0.05.

** Significant at P <0.01.

I = Increase over control.

DOMINANT LETHAL GENE-RAT

Table 12

AVERAGE PREIMPLANTATION LOSS PER PREGNANT FEMALE

Compound: FD&C Red #2
FDA No: 71-23

Week of study	Control (10 ml/kg)	TEM (0.2 mg/kg)	71-23 (30 mg/kg)	71-23 (2.5 g/kg)	71-23 (5 g/kg)
<u>Acute-Single Dose</u>					
1	20/16=1.25	46/15=3.07	29/16=1.81	18/19=0.95	25/18=1.39
2	14/16=0.88	64/15=4.27**	17/12=1.42	40/16=2.50	33/18=1.83
3	12/17=0.71	100/18=5.56**	31/14=2.21	26/16=1.63	11/15=0.73
4	18/18=1.00	118/15=7.87**	34/17=2.00	15/18=0.83	45/16=2.81*
5	23/20=1.15	55/17=3.24	17/16=1.06	15/20=0.75	30/19=1.58
6	11/20=0.55	25/15=1.67	32/13=2.46	21/17=1.24	23/20=1.15
7	26/20=1.30	29/16=1.81	23/15=1.53	22/18=1.22	40/20=2.00
8	11/20=0.55	20/17=1.18	22/15=1.47	36/20=1.80	19/20=0.95
<u>Subacute-Multiple Dose</u>					
1	12/16=0.75		24/12=2.00	16/12=1.33	42/17=2.47
2	9/17=0.53		23/18=1.28	17/18=0.94	24/17=1.35
3	24/17=1.41		29/17=1.71	23/17=1.35	20/16=1.25
4	15/17=0.88		28/20=1.40	36/19=1.89	22/20=1.10
5	16/20=0.80		28/19=1.47	18/20=0.90	11/20=0.55
6	38/20=1.90		38/19=2.00	38/20=1.90	32/20=1.60
7	5/18=0.28		22/20=1.10	5/20=0.25	25/20=1.25

* Significant at P <0.05:

** Significant at P <0.01.

Raw Data and Statistical Analyses

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DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD+C RED NO. 2

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TEST MATERIAL	WEEK	S/M	DOSE	MALE			PREG.	IMPLANTS		EARLY DEATHS		LATE DEATHS		CORPORA LUTEA	
				NO.	NO.	PREG.		L	R	L	R	L	R	L	R
CNTRL23	1	S	0.0000	1	1	Y		6	5	0	0	1	0	7	5
CNTRL23	1	S	0.0000	1	2	N		-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	1	S	0.0000	2	3	Y		4	6	0	0	1	1	5	7
CNTRL23	1	S	0.0000	2	4	YY		11	6	0	0	0	0	13	13
CNTRL23	1	S	0.0000	3	5	Y		4	8	0	0	1	0	5	8
CNTRL23	1	S	0.0000	3	6	YY		7	8	0	0	0	1	7	8
CNTRL23	1	S	0.0000	4	7	Y		7	6	0	0	0	0	8	6
CNTRL23	1	S	0.0000	4	8	Y		7	5	0	0	0	0	8	5
CNTRL23	1	S	0.0000	5	9	YY		6	7	0	0	2	0	6	8
CNTRL23	1	S	0.0000	5	10	N		-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	1	S	0.0000	6	11	NY		-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	1	S	0.0000	6	12	YY		6	8	0	0	0	1	6	8
CNTRL23	1	S	0.0000	7	13	YY		4	9	0	0	1	0	4	9
CNTRL23	1	S	0.0000	7	14	N		-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	1	S	0.0000	8	15	Y		8	4	0	0	0	0	8	5
CNTRL23	1	S	0.0000	8	16	YY		7	8	0	0	0	0	7	8
CNTRL23	1	S	0.0500	9	17	YY		8	4	0	0	2	0	8	4
CNTRL23	1	S	0.0500	9	18	YY		4	7	0	0	1	3	4	9
CNTRL23	1	S	0.0500	10	19	YY		4	6	0	0	0	0	4	6
CNTRL23	1	S	0.0500	10	20	Y		4	8	0	0	0	0	4	9
71-23	1	S	.0300	51	101	Y		7	6	0	0	0	0	7	6
71-23	1	S	.0300	51	102	NY		-0	-0	-0	-0	-0	-0	-0	-0
71-23	1	S	.0300	52	103	YY		0	4	0	0	0	1	4	4
71-23	1	S	.0300	52	104	N		-0	-0	-0	-0	-0	-0	-0	-0
71-23	1	S	.0300	53	105	YY		7	6	0	0	1	0	7	6
71-23	1	S	.0300	53	106	YY		7	7	0	0	0	0	7	7
71-23	1	S	.0300	54	107	N		-0	-0	-0	-0	-0	-0	-0	-0
71-23	1	S	.0300	54	108	NY		-0	-0	-0	-0	-0	-0	-0	-0
71-23	1	S	.0300	55	109	YY		6	7	0	0	0	0	10	8
71-23	1	S	.0300	55	110	YY		6	8	0	0	1	0	6	8
71-23	1	S	.0300	56	111	YY		9	6	0	0	0	0	10	6
71-23	1	S	.0300	56	112	YY		5	5	0	0	1	0	5	7
71-23	1	S	.0300	57	113	YY		7	7	0	0	0	1	6	8
71-23	1	S	.0300	57	114	YY		6	6	0	0	0	0	6	8
71-23	1	S	.0300	58	115	YY		5	8	0	0	1	0	6	8
71-23	1	S	.0300	58	116	YY		5	7	0	0	0	0	6	8
71-23	1	S	.0300	59	117	YY		6	7	0	0	0	1	6	10
71-23	1	S	.0300	59	118	YY		0	6	0	0	0	0	6	11
71-23	1	S	.0300	60	119	YY		6	11	0	0	0	1	6	6
71-23	1	S	.0300	60	120	Y		6	5	0	0	0	0	6	6

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23 FD+C REC NO. 2 PAGE 2

TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS L R	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA L R	
								L	R	L	R	L	R
71-23	1	S	2.5000	61	121	Y	8	5	0	0	1	0	9 5
71-23	1	S	2.5000	61	122	Y	4	9	0	0	0	2	5 9
71-23	1	S	2.5000	62	123	Y	8	4	0	0	1	0	4 9
71-23	1	S	2.5000	62	124	Y	4	8	0	0	0	1	0 5
71-23	1	S	2.5000	63	125	Y	5	9	0	0	0	1	0 5
71-23	1	S	2.5000	63	126	Y	7	4	0	0	0	1	8 4
71-23	1	S	2.5000	64	127	N	-0	-0	-0	-0	-0	-0	-0 -0
71-23	1	S	2.5000	64	128	Y	6	8	0	0	6	6	6 9
71-23	1	S	2.5000	65	129	Y	9	4	0	0	0	0	9 5
71-23	1	S	2.5000	65	130	Y	5	7	0	0	0	1	7 9
71-23	1	S	2.5000	66	131	Y	7	8	0	0	0	1	6 8
71-23	1	S	2.5000	66	132	Y	5	8	0	0	0	1	10 9
71-23	1	S	2.5000	67	133	Y	9	5	0	0	0	0	0 5
71-23	1	S	2.5000	67	134	Y	7	5	0	0	0	0	0 0
71-23	1	S	2.5000	68	135	Y	6	8	0	0	0	0	8 7
71-23	1	S	2.5000	68	136	Y	6	8	0	0	0	0	6 8
71-23	1	S	2.5000	69	137	Y	9	6	0	0	0	1	9 6
71-23	1	S	2.5000	69	138	Y	13	3	0	0	0	1	13 3
71-23	1	S	2.5000	70	139	Y	5	7	0	0	0	1	8 6
71-23	1	S	2.5000	70	140	Y	6	5	0	0	0	0	7 6
71-23	1	S	5.0000	71	141	Y	5	10	0	0	0	0	1 5
71-23	1	S	5.0000	71	142	Y	4	5	0	0	0	0	7 5
71-23	1	S	5.0000	72	143	Y	5	6	0	0	0	0	4 8
71-23	1	S	5.0000	72	144	Y	7	5	0	0	0	2	7 5
71-23	1	S	5.0000	73	145	N	-0	-0	-0	-0	-0	-0	-0 -0
71-23	1	S	5.0000	73	146	Y	4	7	0	0	0	1	6 8
71-23	1	S	5.0000	74	147	Y	6	8	0	0	0	0	6 8
71-23	1	S	5.0000	74	148	Y	6	5	0	0	0	0	6 5
71-23	1	S	5.0000	75	149	Y	4	10	0	0	0	0	4 10
71-23	1	S	5.0000	75	150	Y	8	4	0	0	0	0	8 5
71-23	1	S	5.0000	76	151	Y	3	11	0	0	0	1	3 12
71-23	1	S	5.0000	76	152	Y	8	5	0	0	0	0	8 5
71-23	1	S	5.0000	77	153	Y	7	5	0	0	0	0	7 6
71-23	1	S	5.0000	77	154	Y	4	8	0	0	0	0	4 8
71-23	1	S	5.0000	78	155	N	-0	-0	-0	-0	-0	-0	-0 -0
71-23	1	S	5.0000	78	156	Y	8	3	0	0	0	0	9 3
71-23	1	S	5.0000	79	157	Y	4	8	0	0	0	0	8 5
71-23	1	S	5.0000	79	158	Y	7	5	0	0	0	0	6 9
71-23	1	S	5.0000	80	159	Y	0	0	0	0	0	0	0 0
71-23	1	S	5.0000	80	160	Y	8	6	0	0	0	1	0 10

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD+C RED NO. 2

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TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA		
								L	R	L	R	L	R	
TEM23	1	S	.0002	11	21	Y	7	7	0	0	3	5	7	7
TEM23	1	S	.0002	11	22	YY	4	8	0	0	0	2	5	9
TEM23	1	S	.0002	12	23	YY	6	5	0	0	3	0	6	6
TEM23	1	S	.0002	12	24	YY	3	6	1	0	1	2	4	7
TEM23	1	S	.0002	13	25	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	1	S	.0002	13	26	YY	1	0	1	0	0	0	9	10
TEM23	1	S	.0002	14	27	YY	3	9	0	0	0	3	3	10
TEM23	1	S	.0002	14	28	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	1	S	.0002	15	29	NY	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	1	SS	.0002	15	30	Y	7	3	0	0	1	2	7	5
TEM23	1	S	.0002	16	31	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	1	S	.0002	16	32	YY	5	10	0	0	1	4	13	10
TEM23	1	S	.0002	17	33	YY	6	8	0	0	3	4	6	9
TEM23	1	S	.0002	17	34	YY	6	9	0	0	2	4	8	13
TEM23	1	S	.0002	18	35	YY	7	7	0	0	1	1	7	9
TEM23	1	S	.0002	18	36	YY	8	6	0	0	3	5	9	6
TEM23	1	S	.0002	19	37	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	1	S	.0002	19	38	YY	7	6	0	0	0	0	7	6
TEM23	1	S	.0002	20	39	YY	4	10	0	0	2	5	4	11
TEM23	1	S	.0002	20	40	Y	9	6	0	0	6	5	10	6
CNTRL23	1	M	0.0000	1	1	Y	7	5	0	0	0	1	8	5
CNTRL23	1	M	0.0000	1	2	N	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	1	M	0.0000	2	3	YY	5	9	0	0	0	1	6	9
CNTRL23	1	M	0.0000	2	4	YY	8	5	0	0	0	0	8	6
CNTRL23	1	M	0.0000	3	5	YY	4	7	0	0	0	0	6	6
CNTRL23	1	M	0.0000	3	6	YY	6	9	0	0	4	3	6	9
CNTRL23	1	M	0.0000	4	7	YY	9	6	0	0	1	0	9	6
CNTRL23	1	M	0.0000	4	8	YY	4	9	0	0	1	1	5	9
CNTRL23	1	M	0.0000	5	9	YY	8	6	0	0	2	1	9	6
CNTRL23	1	M	0.0000	5	10	YY	3	9	0	0	0	1	3	9
CNTRL23	1	M	0.0000	6	11	YY	8	4	0	0	0	0	8	6
CNTRL23	1	M	0.0000	6	12	YY	8	6	0	0	0	0	8	6
CNTRL23	1	M	0.0000	7	13	NN	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	1	M	0.0000	7	14	NN	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	1	M	0.0000	8	15	YY	5	10	0	0	0	0	5	10
CNTRL23	1	M	0.0000	8	16	N	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	1	M	0.0000	9	17	YY	10	3	0	0	0	0	10	3
CNTRL23	1	M	0.0000	9	18	YY	8	5	0	0	0	0	8	6
CNTRL23	1	M	0.0000	10	19	YY	3	8	0	0	0	0	3	9
CNTRL23	1	M	0.0000	10	20	Y	5	8	0	0	0	1	6	8

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

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DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD+C RED NO. 2

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TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS		EARLY DEATHS		LATE DEATHS		CORPORA LUTEA	
							L	R	L	R	L	R	L	R
71-23	1	M	5.0000	61	121	Y	8	6	0	0	0	0	9	6
71-23	1	M	5.0000	61	122	YY	3	5	0	0	0	1	5	6
71-23	1	M	5.0000	62	123	YY	5	7	0	0	0	0	6	8
71-23	1	M	5.0000	62	124	YY	6	5	0	0	2	0	7	5
71-23	1	M	5.0000	63	125	YY	4	6	0	0	0	0	4	6
71-23	1	M	5.0000	63	126	YY	2	8	0	0	0	0	6	8
71-23	1	M	5.0000	64	127	YY	6	7	0	0	3	1	6	8
71-23	1	M	5.0000	64	128	YY	5	10	0	0	0	0	5	10
71-23	1	M	5.0000	65	129	YY	8	5	0	0	1	0	8	5
71-23	1	M	5.0000	65	130	YY	4	5	0	0	0	0	5	8
71-23	1	M	5.0000	66	131	YY	4	8	0	0	0	2	4	8
71-23	1	M	5.0000	66	132	NY	-0	-0	-0	-0	-0	-0	-0	-0
71-23	1	M	5.0000	67	133	YY	5	5	0	0	1	0	5	5
71-23	1	M	5.0000	67	134	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	1	M	5.0000	68	135	YY	2	0	0	0	0	0	7	5
71-23	1	M	5.0000	68	136	YY	7	7	0	0	0	0	7	7
71-23	1	M	5.0000	69	137	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	1	M	5.0000	69	138	YY	7	5	0	0	1	0	7	5
71-23	1	M	5.0000	70	139	YY	2	2	0	0	0	0	9	2
71-23	1	M	5.0000	70	140	Y	0	1	0	1	0	0	6	4

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

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TEST MATERIAL	WEEK	S/M	DOSE	MALE		PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA		
				NO.	NO.			L	R	L	R	L	H	
CNTRL23	2	S	0.0000	1	1	Y	3	7	0	0	0	1	4	7
CNTRL23	2	S	0.0000	1	2	N	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	2	S	0.0000	2	3	YY	10	2	0	0	0	0	10	2
CNTRL23	2	S	0.0000	2	4	YY	6	4	0	0	0	0	9	4
CNTRL23	2	S	0.0000	3	5	N	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	2	S	0.0000	3	6	YY	6	9	0	0	0	2	6	9
CNTRL23	2	S	0.0000	4	7	YY	9	5	0	0	1	0	9	6
CNTRL23	2	S	0.0000	4	8	N	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	2	S	0.0000	5	9	YY	3	10	0	0	0	0	3	10
CNTRL23	2	S	0.0000	5	10	YY	7	5	0	0	1	1	8	6
CNTRL23	2	S	0.0000	6	11	YY	6	7	0	0	1	0	6	9
CNTRL23	2	S	0.0000	6	12	YY	3	10	0	0	0	0	3	10
CNTRL23	2	S	0.0000	7	13	YY	5	6	0	0	0	0	5	7
CNTRL23	2	S	0.0000	7	14	YY	8	3	0	0	0	0	8	3
CNTRL23	2	S	0.0000	8	15	YY	7	5	0	0	0	1	7	6
CNTRL23	2	S	0.0000	8	16	YY	8	6	0	0	0	0	9	6
CNTRL23	2	S	0.0000	9	17	YY	7	8	0	0	0	0	7	8
CNTRL23	2	S	0.0000	9	18	N	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	2	S	0.0000	10	19	YY	3	9	0	0	0	0	3	10
CNTRL23	2	S	0.0000	10	20	Y	5	7	0	0	0	1	6	7
71-23	2	S	.0300	51	101	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	2	S	.0300	51	102	NY	-0	-0	-0	-0	-0	-0	-0	-0
71-23	2	S	.0300	52	103	YY	7	8	0	0	0	1	7	8
71-23	2	S	.0300	52	104	Y	6	6	0	0	0	1	6	6
71-23	2	S	.0300	53	105		0	0	0	0	0	0	0	0
71-23	2	S	.0300	53	106		0	0	0	0	0	0	0	0
71-23	2	S	.0300	54	107		0	0	0	0	0	0	0	0
71-23	2	S	.0300	54	108		0	0	0	0	0	0	0	0
71-23	2	S	.0300	55	109	Y	7	6	0	0	0	0	13	8
71-23	2	S	.0300	55	110	YY	6	6	0	0	1	0	7	6
71-23	2	S	.0300	56	111	YY	5	7	0	0	2	3	5	7
71-23	2	S	.0300	56	112	YY	6	7	0	0	0	0	8	8
71-23	2	S	.0300	57	113	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	2	S	.0300	57	114	YY	5	5	0	0	0	0	7	5
71-23	2	S	.0300	58	115	YY	3	10	0	0	0	0	3	10
71-23	2	S	.0300	58	116	YY	6	7	0	0	1	0	7	7
71-23	2	S	.0300	59	117	YY	6	4	0	0	1	1	8	4
71-23	2	S	.0300	59	118	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	2	S	.0300	60	119	YY	9	5	0	0	1	0	9	5
71-23	2	S	.0300	60	120	Y	4	9	0	0	0	0	4	9

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TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS L R	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA L R	
								L	R	L	R	L	R
71-23	2	S	2.5000	61	121	Y	5 5	0	0	0	0	6	7
71-23	2	S	2.5000	61	122	N	-0 -0	-0	-0	-0	-0	-0	-0
71-23	2	S	2.5000	62	123	Y	6 6	0	0	0	1	6	7
71-23	2	S	2.5000	62	124	Y	0 1	0	0	0	0	11	7
71-23	2	S	2.5000	63	125	Y	5 3	0	0	1	0	6	4
71-23	2	S	2.5000	63	126	Y	6 7	0	0	0	1	7	7
71-23	2	S	2.5000	64	127	Y	7 6	0	0	0	0	8	6
71-23	2	S	2.5000	64	128	Y	7 4	0	0	1	0	8	4
71-23	2	S	2.5000	65	129	Y	6 3	0	0	0	0	8	4
71-23	2	S	2.5000	65	130	Y	8 3	0	0	0	0	9	3
71-23	2	S	2.5000	66	131	Y	4 8	0	0	1	0	4	8
71-23	2	S	2.5000	66	132	N	-0 -0	-0	-0	-0	-0	-0	-0
71-23	2	S	2.5000	67	133	Y	5 8	0	0	0	1	5	4
71-23	2	S	2.5000	67	134	N	-0 -0	-0	-0	-0	-0	-0	-0
71-23	2	S	2.5000	68	135	Y	3 8	0	0	0	0	3	8
71-23	2	S	2.5000	68	136	Y	4 6	0	0	0	0	4	7
71-23	2	S	2.5000	69	137	Y	6 7	0	0	0	0	8	7
71-23	2	S	2.5000	69	138	Y	5 7	0	0	0	0	7	11
71-23	2	S	2.5000	70	139	N	-0 -0	-0	-0	-0	-0	-0	-0
71-23	2	S	2.5000	70	140	Y	5 5	0	0	0	0	5	5
71-23	2	S	5.0000	71	141	Y	7 8	0	0	0	0	7	8
71-23	2	S	5.0000	71	142	Y	7 5	0	0	0	0	9	8
71-23	2	S	5.0000	72	143	N	-0 -0	-0	-0	-0	-0	-0	-0
71-23	2	S	5.0000	72	144	Y	4 7	0	0	0	0	4	8
71-23	2	S	5.0000	73	145	Y	5 9	0	0	0	0	5	9
71-23	2	S	5.0000	73	146	Y	7 5	0	0	0	1	7	5
71-23	2	S	5.0000	74	147	Y	10 4	0	0	0	0	10	4
71-23	2	S	5.0000	74	148	Y	6 7	0	0	0	0	9	7
71-23	2	S	5.0000	75	149	N	-0 -0	-0	-0	-0	-0	-0	-0
71-23	2	S	5.0000	75	150	Y	0 9	0	0	0	0	0	9
71-23	2	S	5.0000	76	151	Y	3 6	0	0	0	0	5	8
71-23	2	S	5.0000	76	152	Y	6 5	0	0	0	6	8	6
71-23	2	S	5.0000	77	153	Y	5 8	0	0	0	1	5	8
71-23	2	S	5.0000	77	154	Y	10 4	0	0	0	0	10	4
71-23	2	S	5.0000	78	155	Y	4 6	0	0	0	0	4	6
71-23	2	S	5.0000	78	156	Y	6 5	0	0	0	0	7	5
71-23	2	S	5.0000	79	157	Y	0 2	0	0	0	0	9	3
71-23	2	S	5.0000	79	158	Y	5 8	0	0	0	0	5	8
71-23	2	S	5.0000	80	159	Y	5 6	0	0	0	0	5	7
71-23	2	S	5.0000	80	160	Y	4 3	0	0	0	0	8	4

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TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA		
								L	R	L	R	L	R	
TEM23	2	S	.0002	11	21	Y	5	6	0	0	2	2	5	7
TEM23	2	S	.0002	11	22	YY	3	3	0	0	3	3	7	4
TEM23	2	S	.0002	12	23	YY	3	4	0	0	3	4	8	6
TEM23	2	S	.0002	12	24	YY	5	6	0	0	1	4	5	8
TEM23	2	S	.0002	13	25	YY	6	7	0	0	5	6	6	9
TEM23	2	S	.0002	13	26	NN	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	2	S	.0002	14	27	NN	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	2	S	.0002	14	28	YY	4	6	0	0	3	6	5	7
TEM23	2	S	.0002	15	29	YY	4	4	0	0	3	2	6	5
TEM23	2	S	.0002	15	30	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	2	S	.0002	16	31	Y	1	2	0	0	1	1	7	11
TEM23	2	S	.0002	16	32	YY	2	5	0	0	2	5	3	11
TEM23	2	S	.0002	17	33	YY	3	5	0	0	3	5	4	7
TEM23	2	S	.0002	17	34	YY	3	2	0	0	3	2	4	7
TEM23	2	S	.0002	18	35	YY	7	4	0	0	6	4	7	5
TEM23	2	S	.0002	18	36	YY	5	7	0	0	5	7	5	8
TEM23	2	S	.0002	19	37	YY	3	6	0	0	3	6	3	7
TEM23	2	S	.0002	19	38	NN	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	2	S	.0002	20	39	NY	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	2	S	.0002	20	40	Y	1	2	0	0	1	2	4	7
CNTRL23	2	M	0.0000	1	1	YY	4	11	0	0	0	0	4	11
CNTRL23	2	M	0.0000	1	2	YY	9	4	0	0	0	0	10	4
CNTRL23	2	M	0.0000	2	3	YY	7	5	0	0	3	0	9	5
CNTRL23	2	M	0.0000	2	4	YY	3	9	0	0	0	0	3	9
CNTRL23	2	M	0.0000	3	5	YY	7	5	3	0	0	2	7	5
CNTRL23	2	M	0.0000	3	6	YY	4	6	0	0	0	0	4	7
CNTRL23	2	M	0.0000	4	7	NY	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	2	M	0.0000	4	8	YY	3	10	0	1	0	0	3	11
CNTRL23	2	M	0.0000	5	9	YY	5	10	0	1	0	0	6	10
CNTRL23	2	M	0.0000	5	10	NY	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	2	M	0.0000	6	11	YY	3	9	0	1	0	0	3	10
CNTRL23	2	M	0.0000	6	12	YY	9	6	0	1	0	0	9	6
CNTRL23	2	M	0.0000	7	13	NY	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	2	M	0.0000	7	14	YY	8	4	0	0	0	0	8	4
CNTRL23	2	M	0.0000	8	15	YY	3	12	0	0	0	0	1	12
CNTRL23	2	M	0.0000	8	16	YY	7	5	0	0	0	0	8	5
CNTRL23	2	M	0.0000	9	17	YY	10	4	0	0	0	0	10	4
CNTRL23	2	M	0.0000	9	18	YY	4	10	0	0	0	0	4	10
CNTRL23	2	M	0.0000	10	19	YY	7	5	0	0	1	0	7	6
CNTRL23	2	M	0.0000	10	20	Y	6	7	0	1	0	0	6	7

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TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA	
								L	R	L	R	L	R
71-23	2	M	.0300	41	81	Y	10	3	1	0	0	0	10 3
71-23	2	M	.0300	41	82	YY	9	6	0	0	0	1	9 6
71-23	2	M	.0300	42	83	YY	7	5	0	0	0	0	7 5
71-23	2	M	.0300	42	84	YY	8	5	0	0	0	0	8 5
71-23	2	M	.0300	43	85	YY	3	7	0	0	0	1	6 15
71-23	2	M	.0300	43	86	YY	6	8	0	1	0	0	6 8
71-23	2	M	.0300	44	87	YY	7	6	0	0	0	0	7 6
71-23	2	M	.0300	44	88	YY	3	6	0	0	0	0	3 12
71-23	2	M	.0300	45	89	NY	-0	-0	-0	-0	-0	-0	-0 -0
71-23	2	M	.0300	45	90	Y	6	8	0	0	0	1	6 8
71-23	2	M	.0300	46	91	N	-0	-0	-0	-0	-0	-0	-0 -0
71-23	2	M	.0300	46	92	YY	6	7	0	0	0	0	6 8
71-23	2	M	.0300	47	93	YY	6	4	0	0	0	1	8 6
71-23	2	M	.0300	47	94	YY	5	10	0	1	0	0	5 10
71-23	2	M	.0300	48	95	YY	4	8	0	0	0	1	4 8
71-23	2	M	.0300	48	96	YY	7	6	0	0	0	0	7 6
71-23	2	M	.0300	49	97	YY	6	8	0	0	0	0	6 9
71-23	2	M	.0300	49	98	YY	8	8	0	0	0	1	8 8
71-23	2	M	.0300	50	99	YY	6	8	0	0	0	1	6 8
71-23	2	M	.0300	50	100	Y	10	5	0	0	0	0	10 5
71-23	2	M	2.5000	51	101	YY	6	8	0	0	0	1	6 8
71-23	2	M	2.5000	51	102	YY	7	7	0	0	2	0	8 8
71-23	2	M	2.5000	52	103	YY	9	7	0	0	0	0	9 7
71-23	2	M	2.5000	52	104	YY	7	9	2	0	1	1	7 9
71-23	2	M	2.5000	53	105	YY	8	5	0	0	2	2	9 5
71-23	2	M	2.5000	53	106	YY	8	7	2	0	2	2	8 7
71-23	2	M	2.5000	54	107	YY	8	8	0	1	1	0	9 8
71-23	2	M	2.5000	54	108	YY	5	9	0	0	0	0	5 10
71-23	2	M	2.5000	55	109	YY	2	10	0	0	0	1	3 10
71-23	2	M	2.5000	55	110	YY	4	10	0	0	0	0	4 10
71-23	2	M	2.5000	56	111	YY	7	5	0	0	2	0	7 6
71-23	2	M	2.5000	56	112	YY	9	5	0	0	0	0	9 6
71-23	2	M	2.5000	57	113	YY	6	10	2	0	0	0	6 11
71-23	2	M	2.5000	57	114	NY	-0	-0	-0	-0	-0	-0	-0 -0
71-23	2	M	2.5000	58	115	YY	5	8	0	0	0	0	5 8
71-23	2	M	2.5000	58	116	NY	-0	-0	-0	-0	-0	-0	-0 -0
71-23	2	M	2.5000	59	117	YY	5	7	0	0	0	0	6 8
71-23	2	M	2.5000	59	118	YY	5	7	0	0	0	0	6 7
71-23	2	M	2.5000	60	119	YY	8	6	0	0	3	1	8 6
71-23	2	M	2.5000	60	120	Y	7	6	0	0	0	0	7 7

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TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA		
								L	R	L	R	L	R	
71-23	3	S	2.5000	61	121	Y	7	8	0	0	1	0	7	8
71-23	3	S	2.5000	61	122	YY	5	8	0	0	0	0	5	8
71-23	3	S	2.5000	62	123	YY	6	6	0	0	0	0	7	6
71-23	3	S	2.5000	62	124	YY	5	8	1	0	0	0	5	11
71-23	3	S	2.5000	63	125	YY	4	7	0	0	0	0	10	12
71-23	3	S	2.5000	63	126	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	3	S	2.5000	64	127	YY	8	5	0	0	1	0	8	6
71-23	3	S	2.5000	64	128	NY	-0	-0	-0	-0	-0	-0	-0	-0
71-23	3	S	2.5000	65	129	YY	4	9	0	0	0	2	4	9
71-23	3	S	2.5000	65	130	YY	4	8	0	0	0	0	6	8
71-23	3	S	2.5000	66	131	YY	8	7	0	0	0	0	8	7
71-23	3	S	2.5000	66	132	YY	4	7	0	0	0	0	4	9
71-23	3	S	2.5000	67	133	YY	10	4	0	0	0	1	10	4
71-23	3	S	2.5000	67	134	YY	5	9	0	0	0	0	5	9
71-23	3	S	2.5000	68	135	NY	-0	-0	-0	-0	-0	-0	-0	-0
71-23	3	S	2.5000	68	136	YY	4	7	0	1	0	0	4	9
71-23	3	S	2.5000	69	137	NY	-0	-0	-0	-0	-0	-0	-0	-0
71-23	3	S	2.5000	69	138	YY	5	9	0	0	0	0	5	9
71-23	3	S	2.5000	70	139	YY	4	4	0	0	0	0	4	10
71-23	3	S	2.5000	70	140	Y	4	7	0	0	0	0	4	7
71-23	3	S	5.0000	71	141	YY	7	6	0	1	0	0	7	6
71-23	3	S	5.0000	71	142	YY	5	5	0	0	1	0	5	5
71-23	3	S	5.0000	72	143	NN	-0	-0	-0	-0	-0	-0	-0	-0
71-23	3	S	5.0000	72	144	NNY	-0	-0	-0	-0	-0	-0	-0	-0
71-23	3	S	5.0000	73	145	YY	6	5	0	0	0	0	6	5
71-23	3	S	5.0000	73	146	NY	-0	-0	-0	-0	-0	-0	-0	-0
71-23	3	S	5.0000	74	147	YY	6	5	0	0	0	0	7	5
71-23	3	S	5.0000	74	148	NY	-0	-0	-0	-0	-0	-0	-0	-0
71-23	3	S	5.0000	75	149	YY	6	5	0	0	0	0	6	5
71-23	3	S	5.0000	75	150	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	3	S	5.0000	76	151	YY	4	8	0	0	0	0	4	8
71-23	3	S	5.0000	76	152	YY	8	3	0	0	0	0	8	3
71-23	3	S	5.0000	77	153	YY	9	5	0	0	0	0	9	5
71-23	3	S	5.0000	77	154	YY	5	5	0	0	1	0	8	4
71-23	3	S	5.0000	78	155	YY	5	8	0	0	0	2	8	8
71-23	3	S	5.0000	78	156	YY	7	5	0	0	0	1	8	5
71-23	3	S	5.0000	79	157	YY	6	4	0	0	0	0	7	6
71-23	3	S	5.0000	79	158	YY	6	6	0	0	1	0	8	8
71-23	3	S	5.0000	80	159	YY	5	7	0	0	0	0	7	8
71-23	3	S	5.0000	80	160	Y	7	5	0	0	0	0	8	5

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TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA		
								L	R	L	R	L	R	
TEM23	3	S	.0002	11	21	Y	5	6	1	5	2	1	7	9
TEM23	3	S	.0002	11	22	YY	2	5	0	0	2	5	5	7
TEM23	3	S	.0002	12	23	YY	7	10	3	3	0	0	7	10
TEM23	3	S	.0002	12	24	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	3	S	.0002	13	25	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	3	S	.0002	13	26	YY	5	7	2	6	0	0	5	8
TEM23	3	S	.0002	14	27	YY	1	0	1	0	0	0	15	19
TEM23	3	S	.0002	14	28	YY	9	5	6	4	1	0	9	5
TEM23	3	S	.0002	15	29	YY	2	7	0	0	0	6	3	7
TEM23	3	S	.0002	15	30	YY	6	8	5	7	0	0	6	9
TEM23	3	S	.0002	16	31	YY	4	7	3	6	0	0	5	9
TEM23	3	S	.0002	16	32	YY	5	6	2	3	0	1	6	6
TEM23	3	S	.0002	17	33	YY	7	4	2	1	2	2	7	6
TEM23	3	S	.0002	17	34	YY	6	7	6	6	0	0	7	8
TEM23	3	S	.0002	18	35	YY	6	6	5	5	0	0	5	6
TEM23	3	S	.0002	18	36	YY	5	6	0	0	5	6	7	7
TEM23	3	S	.0002	19	37	YY	1	1	1	1	0	0	7	9
TEM23	3	S	.0002	19	38	YY	2	5	2	5	0	0	2	9
TEM23	3	S	.0002	20	39	YY	2	0	2	0	0	0	10	20
TEM23	3	S	.0002	20	40	Y	5	9	2	7	0	0	5	9
CNTRL23	3	M	0.0000	1	1	N	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	3	M	0.0000	1	2	YY	12	3	0	0	0	0	13	3
CNTRL23	3	M	0.0000	2	3	YY	9	6	0	1	0	0	9	6
CNTRL23	3	M	0.0000	2	4	YY	6	8	0	2	4	4	6	9
CNTRL23	3	M	0.0000	3	5	YY	6	9	1	0	0	0	7	9
CNTRL23	3	M	0.0000	3	6	YY	8	5	0	0	0	0	9	6
CNTRL23	3	M	0.0000	4	7	YY	5	7	0	0	1	1	6	7
CNTRL23	3	M	0.0000	4	8	YY	6	7	2	2	0	2	6	7
CNTRL23	3	M	0.0000	5	9	NY	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	3	M	0.0000	5	10	YY	6	5	0	0	0	0	6	6
CNTRL23	3	M	0.0000	6	11	YY	3	10	0	1	0	0	3	10
CNTRL23	3	M	0.0000	6	12	YY	7	9	0	0	1	1	8	9
CNTRL23	3	M	0.0000	7	13	YY	10	3	0	0	0	0	10	3
CNTRL23	3	M	0.0000	7	14	YY	9	5	0	0	1	0	9	5
CNTRL23	3	M	0.0000	8	15	YY	6	7	0	0	0	0	6	7
CNTRL23	3	M	0.0000	8	16	NY	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	3	M	0.0000	9	17	YY	2	8	1	0	0	0	5	10
CNTRL23	3	M	0.0000	9	18	YY	5	8	0	0	0	0	5	10
CNTRL23	3	M	0.0000	10	19	YY	2	2	0	0	1	0	0	3
CNTRL23	3	M	0.0000	10	20	Y	4	9	0	1	0	0	4	10

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TEST MATERIAL	WEEK	S/M	DOSE	MALE		FEMALE NO.	PREG.	IMPLANTS		EARLY DEATHS		LATE DEATHS		CORPORA LUTEA	
				NO.				L	R	L	R	L	R	L	R
71-23	3	M	5.0000	61	121	Y		10	4	0	0	0	0	10	4
71-23	3	M	5.0000	61	122	Y		5	8	1	0	0	0	5	3
71-23	3	M	5.0000	62	123	YY		7	5	0	0	0	0	7	5
71-23	3	M	5.0000	62	124	YY		8	4	0	0	1	1	8	4
71-23	3	M	5.0000	63	125	YY		8	5	0	0	1	0	9	5
71-23	3	M	5.0000	63	126	YY		5	8	0	0	0	0	5	8
71-23	3	M	5.0000	64	127	N		-0	-0	-0	-0	-0	-0	-0	-0
71-23	3	M	5.0000	64	128	YY		5	5	0	0	0	0	5	5
71-23	3	M	5.0000	65	129	YY		5	7	0	1	0	0	5	7
71-23	3	M	5.0000	65	130	YY		9	1	0	0	0	0	10	1
71-23	3	M	5.0000	66	131	YY		0	2	0	0	0	0	5	10
71-23	3	M	5.0000	66	132	NN		-0	-0	-0	-0	-0	-0	-0	-0
71-23	3	M	5.0000	67	133	N		-0	-0	-0	-0	-0	-0	-0	-0
71-23	3	M	5.0000	67	134	YY		10	8	1	0	1	0	10	8
71-23	3	M	5.0000	68	135	NY		-0	-0	-0	-0	-0	-0	-0	-0
71-23	3	M	5.0000	68	136	YY		8	4	1	1	0	0	10	4
71-23	3	M	5.0000	69	137	YY		4	5	0	0	0	0	7	5
71-23	3	M	5.0000	69	138	YY		8	5	0	0	0	0	8	5
71-23	3	M	5.0000	70	139	YY		8	3	0	0	0	0	8	3
71-23	3	M	5.0000	70	140	Y		9	5	1	0	0	0	9	5

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FD+C RED NO. 2

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TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA		
								L	R	L	R	L	R	
CNTRL23	4	S	0.0000	1	1	Y	6	7	0	0	0	0	5	7
CNTRL23	4	S	0.0000	1	2	YY	5	6	0	0	1	0	1	7
CNTRL23	4	S	0.0000	2	3	YY	9	4	1	1	0	0	4	4
CNTRL23	4	S	0.0000	2	4	YY	6	5	0	1	0	0	5	5
CNTRL23	4	S	0.0000	3	5	YY	8	5	0	0	1	0	2	12
CNTRL23	4	S	0.0000	3	6	YY	2	11	0	0	0	0	4	8
CNTRL23	4	S	0.0000	4	7	YY	1	6	0	0	0	0	-0	-0
CNTRL23	4	S	0.0000	4	8	N	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	4	S	0.0000	5	9	YY	7	8	0	0	0	1	7	8
CNTRL23	4	S	0.0000	5	10	YY	6	8	1	1	0	0	6	8
CNTRL23	4	S	0.0000	6	11	YY	7	4	2	0	0	0	12	4
CNTRL23	4	S	0.0000	6	12	YY	11	4	0	0	0	0	4	8
CNTRL23	4	S	0.0000	7	13	YY	4	8	1	0	0	0	6	9
CNTRL23	4	S	0.0000	7	14	YY	6	9	0	0	1	0	7	5
CNTRL23	4	S	0.0000	8	15	YY	6	5	0	0	0	0	9	7
CNTRL23	4	S	0.0000	8	16	YY	7	6	1	1	0	0	7	5
CNTRL23	4	S	0.0000	9	17	YY	7	5	0	0	0	0	8	10
CNTRL23	4	S	0.0000	9	18	YY	7	8	0	0	0	0	-0	-0
CNTRL23	4	S	0.0000	10	19	NY	-0	-0	-0	-0	-0	-0	7	7
CNTRL23	4	S	0.0000	10	20	Y	7	7	0	2	0	0	7	7
71-23	4	S	.0300	51	101	Y	0	1	0	0	0	0	7	7
71-23	4	S	.0300	51	102	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	4	S	.0300	52	103	YY	9	6	0	0	0	0	10	4
71-23	4	S	.0300	52	104	YY	8	5	0	0	1	0	10	7
71-23	4	S	.0300	53	105	YY	5	6	2	1	1	1	6	7
71-23	4	S	.0300	53	106	YY	6	7	0	1	1	3	-0	-0
71-23	4	S	.0300	54	107	NN	-0	-0	-0	-0	-0	-0	-0	-0
71-23	4	S	.0300	54	108	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	4	S	.0300	55	109	Y	6	6	0	0	0	0	6	6
71-23	4	S	.0300	55	110	YY	7	4	0	0	0	0	7	8
71-23	4	S	.0300	56	111	YY	7	5	0	0	0	1	8	5
71-23	4	S	.0300	56	112	YY	7	4	1	0	0	1	5	10
71-23	4	S	.0300	57	113	YY	5	10	0	0	0	0	7	8
71-23	4	S	.0300	57	114	YY	7	8	0	0	1	0	4	8
71-23	4	S	.0300	58	115	YY	4	8	0	1	0	0	6	12
71-23	4	S	.0300	58	116	YY	6	2	0	0	2	0	6	9
71-23	4	S	.0300	59	117	YY	6	8	0	0	0	0	5	7
71-23	4	S	.0300	59	118	YY	5	7	1	1	0	0	3	4
71-23	4	S	.0300	60	119	YY	6	4	0	1	0	0	7	1
71-23	4	S	.0300	60	120	Y	7	7	0	0	0	0		

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD+C RED NO. 2

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TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA	
								L	R	L	R	L	R
71-23	4	S	2.5000	61	121	N	-0	-0	-0	-0	-0	-0	-0
71-23	4	S	2.5000	61	122	YY	7	5	0	0	0	0	7
71-23	4	S	2.5000	62	123	YY	8	5	0	0	0	0	5
71-23	4	S	2.5000	62	124	YY	8	5	0	3	0	0	5
71-23	4	S	2.5000	63	125	NY	-0	-0	-0	-0	-0	-0	-0
71-23	4	S	2.5000	63	126	YY	5	6	0	0	0	0	6
71-23	4	S	2.5000	64	127	YY	4	9	0	2	0	0	5
71-23	4	S	2.5000	64	128	YY	5	9	0	0	0	0	6
71-23	4	S	2.5000	65	129	YY	6	5	0	1	0	0	7
71-23	4	S	2.5000	65	130	YY	5	7	0	0	0	0	7
71-23	4	S	2.5000	66	131	YY	7	6	0	0	0	0	6
71-23	4	S	2.5000	66	132	YY	6	6	0	0	0	0	6
71-23	4	S	2.5000	67	133	YY	5	6	0	1	0	0	7
71-23	4	S	2.5000	67	134	YY	3	9	1	2	0	0	11
71-23	4	S	2.5000	68	135	YY	8	5	0	0	0	0	5
71-23	4	S	2.5000	68	136	YY	5	8	0	0	0	0	5
71-23	4	S	2.5000	69	137	YY	8	8	0	0	4	3	8
71-23	4	S	2.5000	69	138	YY	9	6	0	0	0	1	7
71-23	4	S	2.5000	70	139	YY	7	7	1	0	0	0	4
71-23	4	S	2.5000	70	140	YY	4	6	1	0	0	2	7
71-23	4	S	5.0000	71	141	YY	7	5	0	1	0	0	9
71-23	4	S	5.0000	71	142	YY	5	5	0	0	0	0	6
71-23	4	S	5.0000	72	143	YY	4	7	1	1	0	0	6
71-23	4	S	5.0000	72	144	YY	6	6	0	0	0	0	5
71-23	4	S	5.0000	73	145	YY	8	8	0	0	0	0	8
71-23	4	S	5.0000	73	146	NY	-0	-0	-0	-0	-0	-0	-0
71-23	4	S	5.0000	74	147	NY	-0	-0	-0	-0	-0	-0	-0
71-23	4	S	5.0000	74	148	YY	4	4	2	2	0	0	6
71-23	4	S	5.0000	75	149	YY	4	6	0	0	0	0	4
71-23	4	S	5.0000	75	150	YY	2	2	2	2	0	0	4
71-23	4	S	5.0000	76	151	NY	-0	-0	-0	-0	-0	-0	10
71-23	4	S	5.0000	76	152	YY	9	5	0	1	0	0	5
71-23	4	S	5.0000	77	153	YY	0	1	0	0	0	0	4
71-23	4	S	5.0000	77	154	YY	8	6	0	0	0	1	6
71-23	4	S	5.0000	78	155	YY	7	5	0	0	0	0	5
71-23	4	S	5.0000	78	156	NY	-0	-0	-0	-0	-0	-0	6
71-23	4	S	5.0000	79	157	YY	6	6	1	0	0	0	7
71-23	4	S	5.0000	79	158	YY	7	5	0	1	0	0	7
71-23	4	S	5.0000	80	159	YY	6	6	1	0	0	0	8
71-23	4	S	5.0000	80	160	YY	7	5	0	0	0	0	7

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

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TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA		
								L	R	L	R	L	R	
TEM23	4	S	.0002	11	21	Y	5	5	0	0	3	5	5	9
TEM23	4	S	.0002	11	22	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	4	S	.0002	12	23	YY	4	5	4	4	0	0	7	7
TEM23	4	S	.0002	12	24	YY	2	3	2	2	0	0	5	6
TEM23	4	S	.0002	13	25	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	4	S	.0002	13	26	YY	2	2	1	1	1	1	6	7
TEM23	4	S	.0002	14	27	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	4	S	.0002	14	28	YY	2	3	2	2	0	1	5	8
TEM23	4	S	.0002	15	29	YY	4	1	0	0	4	0	6	5
TEM23	4	S	.0002	15	30	YY	3	2	3	2	0	0	7	7
TEM23	4	S	.0002	16	31	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	4	S	.0002	16	32	YY	2	3	2	3	0	0	6	5
TEM23	4	S	.0002	17	33	YY	4	5	4	5	0	0	5	9
TEM23	4	S	.0002	17	34	YY	1	3	1	3	0	0	5	7
TEM23	4	S	.0002	18	35	YY	3	0	3	0	0	0	7	5
TEM23	4	S	.0002	18	36	YY	3	2	3	2	0	0	8	3
TEM23	4	S	.0002	19	37	YY	0	1	0	1	0	0	2	4
TEM23	4	S	.0002	19	38	YY	1	2	0	2	0	0	9	6
TEM23	4	S	.0002	20	39	YY	0	1	0	1	0	0	-0	-0
TEM23	4	S	.0002	20	40	N	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	4	M	0.0000	1	1	YY	7	6	0	1	0	0	7	6
CNTRL23	4	M	0.0000	1	2	YY	4	7	0	0	0	0	4	9
CNTRL23	4	M	0.0000	2	3	YY	4	8	0	0	0	0	5	8
CNTRL23	4	M	0.0000	2	4	YY	4	10	0	0	0	0	4	10
CNTRL23	4	M	0.0000	3	5	YY	5	9	0	0	0	0	5	10
CNTRL23	4	M	0.0000	3	6	YY	9	8	0	0	0	0	10	8
CNTRL23	4	M	0.0000	4	7	N	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	4	M	0.0000	4	8	NY	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	4	M	0.0000	5	9	YY	8	5	1	0	0	0	8	5
CNTRL23	4	M	0.0000	5	10	YY	7	6	0	0	0	0	7	6
CNTRL23	4	M	0.0000	6	11	YY	5	8	0	0	0	0	6	8
CNTRL23	4	M	0.0000	6	12	YY	4	8	0	0	0	0	4	8
CNTRL23	4	M	0.0000	7	13	YY	8	5	0	0	1	0	3	5
CNTRL23	4	M	0.0000	7	14	YY	8	3	0	0	0	0	-0	-0
CNTRL23	4	M	0.0000	8	15	N	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	4	M	0.0000	8	16	YY	8	5	0	0	0	0	3	6
CNTRL23	4	M	0.0000	9	17	YY	9	3	0	0	1	0	10	3
CNTRL23	4	M	0.0000	9	18	YY	6	8	0	0	0	0	10	11
CNTRL23	4	M	0.0000	10	19	YY	6	7	0	0	0	0	6	7

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD+C RED NO. 2

PAGE 14

TEST MATERIAL	WEEK	S/M	DOSE	MALE			PREG.	IMPLANTS	EARLY		LATE		CORPORA LUTEA		
				NO.	FEMALE NO.	DEATHS			L	R	L	R	L	H	
71-23	4	M	.0300	41	81	Y		6	7	0	0	0	0	6	7
71-23	4	M	.0300	41	82	YY		9	5	0	0	1	0	9	5
71-23	4	M	.0300	42	83	YY		3	7	1	0	0	0	3	8
71-23	4	M	.0300	42	84	YY		8	5	1	0	0	0	8	5
71-23	4	M	.0300	43	85	YY		6	4	0	0	0	0	7	4
71-23	4	M	.0300	43	86	YY		7	8	1	0	0	0	7	8
71-23	4	M	.0300	44	87	YY		7	8	0	0	0	1	7	8
71-23	4	M	.0300	44	88	YY		6	8	0	0	0	0	6	6
71-23	4	M	.0300	45	89	YY		6	6	0	1	1	0	6	7
71-23	4	M	.0300	45	90	YY		6	6	0	1	1	0	5	7
71-23	4	M	.0300	46	91	YY		5	7	1	0	0	0	12	6
71-23	4	M	.0300	46	92	YY		8	1	1	0	0	0	10	6
71-23	4	M	.0300	47	93	YY		10	6	0	0	0	1	8	6
71-23	4	M	.0300	47	94	YY		8	5	0	0	0	1	12	4
71-23	4	M	.0300	48	95	YY		11	3	0	0	0	1	9	4
71-23	4	M	.0300	48	96	YY		8	4	0	0	0	0	6	8
71-23	4	M	.0300	49	97	YY		5	7	0	0	0	0	9	6
71-23	4	M	.0300	49	98	YY		7	5	0	0	0	0	8	5
71-23	4	M	.0300	50	99	YY		8	5	0	0	0	0	4	7
71-23	4	M	.0300	50	100	Y		4	0	0	0	0	0		
71-23	4	M	2.5000	51	101	YY		10	6	2	0	0	0	10	6
71-23	4	M	2.5000	51	102	YY		8	5	0	0	0	0	10	8
71-23	4	M	2.5000	52	103	YY		1	0	1	2	2	0	5	8
71-23	4	M	2.5000	52	104	YY		8	5	0	0	0	0	8	9
71-23	4	M	2.5000	53	105	YY		6	9	0	0	0	1	6	3
71-23	4	M	2.5000	53	106	YY		12	3	0	0	1	1	12	3
71-23	4	M	2.5000	54	107	YY		8	7	0	0	0	0	10	10
71-23	4	M	2.5000	54	108	YY		0	13	0	1	0	0	3	14
71-23	4	M	2.5000	55	109	YY		6	6	0	0	0	1	5	6
71-23	4	M	2.5000	55	110	YY		4	10	0	0	3	0	2	12
71-23	4	M	2.5000	56	111	YY		2	10	0	0	0	0	2	11
71-23	4	M	2.5000	56	112	YY		8	7	0	0	0	0	3	8
71-23	4	M	2.5000	57	113	YY		1	11	0	0	0	0	1	11
71-23	4	M	2.5000	57	114	YY		8	6	0	0	1	0	5	6
71-23	4	M	2.5000	58	115	YY		6	4	0	0	0	0	-0	4
71-23	4	M	2.5000	58	116	Y		-0	-0	-0	-0	-0	-0	7	7
71-23	4	M	2.5000	59	117	YY		5	6	0	0	0	0	6	8
71-23	4	M	2.5000	59	118	YY		6	7	0	1	0	0	3	10
71-23	4	M	2.5000	60	119	YY		3	10	0	0	0	0	6	6
71-23	4	M	2.5000	60	120	Y		6	6	0	0	0	0		

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD+C REF. NO. 2

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TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS		EARLY DEATHS		LATE DEATHS		CORPORA LUTEA	
							L	R	L	R	L	R	L	R
71-23	4	M	5.0000	61	121	Y	5	9	0	0	0	1	5	9
71-23	4	M	5.0000	61	122	Y	5	8	0	0	0	0	5	8
71-23	4	M	5.0000	62	123	Y	4	9	0	0	0	0	4	9
71-23	4	M	5.0000	62	124	Y	4	7	0	0	0	0	4	7
71-23	4	M	5.0000	63	125	Y	9	7	0	0	0	0	9	7
71-23	4	M	5.0000	63	126	Y	4	0	0	0	0	0	5	10
71-23	4	M	5.0000	64	127	Y	7	4	0	0	0	0	7	6
71-23	4	M	5.0000	64	128	Y	4	7	1	0	0	0	4	9
71-23	4	M	5.0000	65	129	Y	5	6	0	0	0	0	5	7
71-23	4	M	5.0000	65	130	Y	5	9	0	0	1	1	7	9
71-23	4	M	5.0000	66	131	Y	5	5	0	0	3	3	5	5
71-23	4	M	5.0000	66	132	Y	6	6	0	0	0	0	6	6
71-23	4	M	5.0000	67	133	Y	7	5	1	0	0	0	7	5
71-23	4	M	5.0000	67	134	Y	7	6	0	0	0	0	7	6
71-23	4	M	5.0000	68	135	Y	2	10	0	0	0	0	2	10
71-23	4	M	5.0000	68	136	Y	1	7	1	0	0	0	7	7
71-23	4	M	5.0000	69	137	Y	8	4	0	0	0	0	11	4
71-23	4	M	5.0000	69	138	Y	4	4	0	0	0	0	4	4
71-23	4	M	5.0000	70	139	Y	5	7	0	0	0	0	5	7
71-23	4	M	5.0000	70	140	Y	5	6	0	0	0	0	6	6

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD+C REG NO. 2

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TEST MATERIAL	WEEK	S/M	DOSE	MALE			PREG.	IMPLANTS		EARLY DEATHS		LATE DEATHS		CORPORA LUTEA	
				NO.	FEMALE NO.	L		L	R	L	R	L	R	L	R
CNTRL23	5	S	0.0000	1	1	Y		4	9	0	0	2	0	4	9
CNTRL23	5	S	0.0000	1	2	Y		4	9	0	0	1	0	4	9
CNTRL23	5	S	0.0000	2	3	Y		4	9	0	0	0	0	4	10
CNTRL23	5	S	0.0000	2	4	Y		4	6	0	0	0	0	5	9
CNTRL23	5	S	0.0000	3	5	Y		8	4	0	0	0	0	9	4
CNTRL23	5	S	0.0000	3	6	Y		9	2	1	0	0	0	10	2
CNTRL23	5	S	0.0000	4	7	Y		2	8	0	0	0	0	3	8
CNTRL23	5	S	0.0000	4	8	Y		4	6	0	0	0	0	7	6
CNTRL23	5	S	0.0000	5	9	Y		3	9	0	0	0	0	3	9
CNTRL23	5	S	0.0000	5	10	Y		5	7	1	0	0	1	6	7
CNTRL23	5	S	0.0000	6	11	Y		8	4	0	0	0	0	9	4
CNTRL23	5	S	0.0000	6	12	Y		3	6	1	0	0	0	6	7
CNTRL23	5	S	0.0000	7	13	Y		6	4	0	0	0	0	9	6
CNTRL23	5	S	0.0000	7	14	Y		8	3	0	0	0	0	6	10
CNTRL23	5	S	0.0000	8	15	Y		6	8	0	0	0	0	5	6
CNTRL23	5	S	0.0000	8	16	Y		5	6	1	2	0	0	6	6
CNTRL23	5	S	0.0000	9	17	Y		6	5	0	0	1	1	6	6
CNTRL23	5	S	0.0000	9	18	Y		6	6	0	0	0	0	6	6
CNTRL23	5	S	0.0000	10	19	Y		5	8	0	0	0	0	5	8
CNTRL23	5	S	0.0000	10	20	Y		7	7	0	0	0	0	7	7
71-23	5	S	.0300	51	101	Y		7	4	0	0	0	0	7	8
71-23	5	S	.0300	51	102	Y		5	10	0	0	0	1	5	11
71-23	5	S	.0300	52	103	Y		5	10	0	0	0	0	5	11
71-23	5	S	.0300	52	104	N		-0	-0	-0	-0	-0	-0	-0	-0
71-23	5	S	.0300	53	105	Y		7	6	0	0	1	2	7	6
71-23	5	S	.0300	53	106	Y		9	4	0	0	0	0	9	5
71-23	5	S	.0300	54	107	Y		-0	-0	-0	-0	-0	-0	-0	-0
71-23	5	S	.0300	54	108	Y		3	8	0	0	0	0	3	8
71-23	5	S	.0300	55	109	Y		5	12	0	0	1	2	5	12
71-23	5	S	.0300	55	110	Y		7	5	0	0	-0	-0	9	9
71-23	5	S	.0300	56	111	N		-0	-0	-0	-0	-0	-0	-0	-0
71-23	5	S	.0300	56	112	N		-0	-0	-0	-0	-0	-0	-0	-0
71-23	5	S	.0300	57	113	Y		9	2	0	0	0	0	9	2
71-23	5	S	.0300	57	114	Y		7	4	1	0	0	0	7	4
71-23	5	S	.0300	58	115	Y		6	9	0	0	0	0	6	9
71-23	5	S	.0300	58	116	Y		6	9	0	0	0	0	6	10
71-23	5	S	.0300	59	117	Y		12	3	0	1	0	0	13	5
71-23	5	S	.0300	59	118	Y		7	5	1	1	0	0	10	5
71-23	5	S	.0300	60	119	Y		6	9	0	2	0	0	7	6
71-23	5	S	.0300	60	120	Y		6	9	0	2	0	0	6	9

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD+C RED NO. 2

PAGE 23

TFST MATERIAL	WEEK	S/M	DOSE	MALE			PREG.	IMPLANTS		EARLY DEATHS		LATE DEATHS		CORPORA LUTEA	
				NU.	FEMALE NO.	L		L	R	L	R	L	R	L	R
TEM23	5	S	.0002	11	21	Y		11	6	1	0	7	6	11	8
TEM23	5	S	.0002	11	22	YY		9	4	0	0	0	1	9	4
TEM23	5	S	.0002	12	23	YY		6	4	0	0	1	1	6	6
TEM23	5	S	.0002	12	24	YY		8	6	0	0	1	1	0	6
TEM23	5	S	.0002	13	25	YY		1	0	0	0	1	0	5	7
TEM23	5	S	.0002	13	26	YY		8	5	0	0	1	3	8	5
TEM23	5	S	.0002	14	27	YY		6	6	0	6	1	0	7	6
TEM23	5	S	.0002	14	28	YY		6	9	0	5	0	0	6	7
TEM23	5	S	.0002	15	29	YY		5	5	3	5	0	0	6	7
TEM23	5	S	.0002	15	30	YY		5	2	3	1	1	0	11	5
TEM23	5	S	.0002	16	31	YY		11	4	0	0	-1	0	-0	-0
TEM23	5	S	.0002	16	32	N		-0	-0	-0	-0	-0	-0	-0	-0
TEM23	5	S	.0002	17	33	YY		6	2	0	0	3	1	9	6
TEM23	5	S	.0002	17	34	YY		2	1	1	0	2	3	8	8
TEM23	5	S	.0002	18	35	YY		7	8	0	0	0	0	7	8
TEM23	5	S	.0002	18	36	YY		4	1	1	1	0	0	4	9
TEM23	5	S	.0002	19	37	YY		7	7	0	0	4	0	7	7
TEM23	5	S	.0002	19	38	YY		6	8	5	3	0	0	6	9
TEM23	5	S	.0002	20	39	NN		-0	-0	-0	-0	-0	-0	-0	-0
TEM23	5	S	.0002	20	40	N		-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	5	M	0.0000	1	1	Y		5	8	0	0	0	0	5	8
CNTRL23	5	M	0.0000	1	2	YY		5	8	0	0	0	0	5	9
CNTRL23	5	M	0.0000	2	3	YY		2	9	0	0	0	0	3	9
CNTRL23	5	M	0.0000	2	4	YY		6	3	0	0	0	0	7	3
CNTRL23	5	M	0.0000	3	5	YY		7	4	0	0	0	0	7	5
CNTRL23	5	M	0.0000	3	6	YY		7	4	0	0	0	0	7	5
CNTRL23	5	M	0.0000	4	7	YY		7	4	0	0	0	0	4	10
CNTRL23	5	M	0.0000	4	8	YY		4	8	0	1	0	0	6	7
CNTRL23	5	M	0.0000	5	9	YY		8	7	0	0	0	0	6	7
CNTRL23	5	M	0.0000	5	10	YY		4	7	0	0	0	0	4	7
CNTRL23	5	M	0.0000	6	11	YY		6	6	0	0	0	0	7	6
CNTRL23	5	M	0.0000	6	12	YY		7	7	0	0	0	0	7	7
CNTRL23	5	M	0.0000	7	13	YY		9	7	0	1	1	0	7	5
CNTRL23	5	M	0.0000	7	14	YY		7	5	0	0	0	0	6	7
CNTRL23	5	M	0.0000	8	15	YY		5	7	0	0	0	0	7	6
CNTRL23	5	M	0.0000	8	16	YY		7	5	0	0	0	0	2	0
CNTRL23	5	M	0.0000	9	17	YY		4	6	0	0	0	0	9	10
CNTRL23	5	M	0.0000	9	18	YY		7	8	0	0	0	0	5	9
CNTRL23	5	M	0.0000	10	19	YY		5	9	0	0	0	0	6	7
CNTRL23	5	M	0.0000	10	20	YY		5	7	0	0	0	0	6	7

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD+C RED NO. 2

PAGE 24

TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA		
								L	R	L	R	L	R	
71-23	5	M	.0300	41	81	Y	2	8	0	0	0	0	5	8
71-23	5	M	.0300	41	82	Y	5	7	0	0	0	0	6	7
71-23	5	M	.0300	42	83	Y	3	8	0	0	0	0	3	9
71-23	5	M	.0300	42	84	Y	5	6	0	0	0	0	5	6
71-23	5	M	.0300	43	85	Y	5	8	0	0	0	0	5	8
71-23	5	M	.0300	43	86	Y	6	8	0	0	1	0	10	13
71-23	5	M	.0300	44	87	Y	10	5	0	0	0	0	10	5
71-23	5	M	.0300	44	88	Y	5	7	0	0	0	0	6	7
71-23	5	M	.0300	45	89	Y	4	8	0	0	1	0	4	8
71-23	5	M	.0300	45	90	Y	6	4	0	1	0	0	7	5
71-23	5	M	.0300	46	91	Y	4	7	0	0	0	0	4	7
71-23	5	M	.0300	46	92	Y	8	5	0	0	0	0	8	5
71-23	5	M	.0300	47	93	Y	9	4	0	0	0	0	9	4
71-23	5	M	.0300	47	94	Y	8	4	0	0	0	1	8	6
71-23	5	M	.0300	48	95	Y	1	5	0	0	0	0	-0	-0
71-23	5	M	.0300	48	96	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	5	M	.0300	49	97	Y	7	7	0	0	0	1	5	8
71-23	5	M	.0300	49	98	Y	4	6	0	0	0	0	6	9
71-23	5	M	.0300	50	99	Y	6	9	0	0	0	0	6	8
71-23	5	M	.0300	50	100	Y	6	7	0	1	0	0	6	8
71-23	5	M	2.5000	51	101	Y	4	7	0	0	0	0	4	7
71-23	5	M	2.5000	51	102	Y	5	7	0	0	0	1	5	3
71-23	5	M	2.5000	52	103	Y	8	3	1	0	1	1	6	7
71-23	5	M	2.5000	52	104	Y	6	7	0	0	0	0	7	7
71-23	5	M	2.5000	53	105	Y	6	6	0	0	0	0	7	6
71-23	5	M	2.5000	53	106	Y	8	4	0	0	0	1	8	4
71-23	5	M	2.5000	54	107	Y	8	4	0	0	0	0	8	2
71-23	5	M	2.5000	54	108	Y	1	9	0	0	0	0	2	9
71-23	5	M	2.5000	55	109	Y	5	8	0	0	0	1	5	8
71-23	5	M	2.5000	55	110	Y	5	8	0	0	0	0	5	3
71-23	5	M	2.5000	56	111	Y	8	3	2	0	0	0	7	8
71-23	5	M	2.5000	56	112	Y	7	7	0	0	0	0	6	5
71-23	5	M	2.5000	57	113	Y	6	5	0	0	0	0	5	7
71-23	5	M	2.5000	57	114	Y	4	7	0	1	0	0	4	8
71-23	5	M	2.5000	58	115	Y	1	3	0	0	0	1	3	8
71-23	5	M	2.5000	58	116	Y	2	8	0	0	0	0	6	7
71-23	5	M	2.5000	59	117	Y	6	7	0	0	0	1	2	0
71-23	5	M	2.5000	59	118	Y	9	3	0	0	0	0	0	0
71-23	5	M	2.5000	60	119	Y	3	7	0	0	0	1	0	0
71-23	5	M	2.5000	60	120	Y	5	8	0	0	0	1	0	0

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD+C RED NO. 2

PAGE 25

TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS		EARLY DEATHS		LATE DEATHS		CORPORA LUTEA	
							L	R	L	R	L	R	L	R
71-23	5	M	5.0000	61	121	Y	7	5	0	0	0	0	7	5
71-23	5	M	5.0000	61	122	Y	4	7	0	0	1	0	4	7
71-23	5	M	5.0000	62	123	Y	5	7	0	0	0	0	5	7
71-23	5	M	5.0000	62	124	Y	6	8	0	0	0	0	6	8
71-23	5	M	5.0000	63	125	Y	6	8	0	0	0	0	5	9
71-23	5	M	5.0000	63	126	Y	13	1	0	0	0	0	13	1
71-23	5	M	5.0000	64	127	Y	5	6	0	0	0	0	5	7
71-23	5	M	5.0000	64	128	Y	5	0	0	0	0	0	6	5
71-23	5	M	5.0000	65	129	Y	3	8	0	0	0	0	4	8
71-23	5	M	5.0000	65	130	Y	6	8	0	0	0	0	6	8
71-23	5	M	5.0000	66	131	Y	7	3	0	0	0	0	7	3
71-23	5	M	5.0000	66	132	Y	6	9	1	0	0	0	7	9
71-23	5	M	5.0000	67	133	Y	5	10	0	0	0	0	5	10
71-23	5	M	5.0000	67	134	Y	6	4	0	0	0	0	7	4
71-23	5	M	5.0000	68	135	Y	7	3	0	0	2	0	7	3
71-23	5	M	5.0000	68	136	Y	3	7	0	1	0	0	3	8
71-23	5	M	5.0000	69	137	Y	8	7	0	0	2	4	8	7
71-23	5	M	5.0000	69	138	Y	6	7	0	0	0	0	6	7
71-23	5	M	5.0000	70	139	Y	9	6	1	1	0	1	2	9
71-23	5	M	5.0000	70	140	Y	9	6	1	1	0	0	9	6

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD-C RED NO. 2

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TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA		
								L	H	L	R	L	H	
CNTRL23	6	S	0.0000	1	1	Y	3	6	0	0	0	0	3	6
CNTRL23	6	S	0.0000	1	2	Y	3	8	0	0	0	0	4	4
CNTRL23	6	S	0.0000	2	3	Y	4	7	0	0	0	0	4	7
CNTRL23	6	S	0.0000	2	4	Y	9	4	0	0	0	0	9	4
CNTRL23	6	S	0.0000	3	5	Y	4	11	0	0	0	0	4	11
CNTRL23	6	S	0.0000	3	6	Y	6	6	0	0	0	0	7	6
CNTRL23	6	S	0.0000	4	7	Y	7	5	0	0	0	0	7	5
CNTRL23	6	S	0.0000	4	8	Y	8	7	0	0	0	0	8	7
CNTRL23	6	S	0.0000	5	9	Y	2	6	0	0	0	0	2	4
CNTRL23	6	S	0.0000	5	10	Y	8	5	0	0	0	0	8	5
CNTRL23	6	S	0.0000	6	11	Y	3	8	0	0	0	0	3	8
CNTRL23	6	S	0.0000	6	12	Y	4	9	0	0	0	0	4	9
CNTRL23	6	S	0.0000	7	13	Y	5	5	0	1	0	0	5	6
CNTRL23	6	S	0.0000	7	14	Y	7	5	0	0	0	0	7	7
CNTRL23	6	S	0.0000	8	15	Y	8	3	0	0	1	0	9	3
CNTRL23	6	S	0.0000	8	16	Y	5	7	0	0	1	0	5	7
CNTRL23	6	S	0.0000	9	17	Y	6	5	0	0	0	0	6	5
CNTRL23	6	S	0.0000	9	18	Y	7	7	0	0	0	0	7	7
CNTRL23	6	S	0.0000	10	19	Y	5	3	0	0	0	1	7	4
CNTRL23	6	S	0.0000	10	20	Y	4	9	0	1	0	0	4	9
71-23	6	S	.0300	51	101	Y	4	6	4	0	0	0	4	7
71-23	6	S	.0300	51	102	Y	0	1	0	0	0	1	4	5
71-23	6	S	.0300	52	103	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	6	S	.0300	52	104	Y	8	6	0	0	0	1	8	6
71-23	6	S	.0300	53	105	Y	4	8	2	0	0	0	5	8
71-23	6	S	.0300	53	106	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	6	S	.0300	54	107	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	6	S	.0300	54	108	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	6	S	.0300	55	109	Y	8	4	0	0	0	0	8	4
71-23	6	S	.0300	55	110	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	6	S	.0300	56	111	Y	4	6	1	0	0	0	5	6
71-23	6	S	.0300	56	112	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	6	S	.0300	57	113	Y	4	7	0	0	0	0	4	7
71-23	6	S	.0300	57	114	Y	5	6	1	0	0	1	10	9
71-23	6	S	.0300	58	115	Y	4	9	0	0	0	0	4	9
71-23	6	S	.0300	58	116	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	6	S	.0300	59	117	Y	4	8	0	0	0	0	7	7
71-23	6	S	.0300	59	118	Y	7	7	0	0	0	0	6	6
71-23	6	S	.0300	60	119	Y	6	6	0	0	0	0	4	4
71-23	6	S	.0300	60	120	Y	0	1	0	0	0	0	4	4

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD-C RED NO. 2

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DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD-C REG NO. 2

PAGE 24

TEST MATERIAL	WEEK	S/M	DOSE	MALE	FEMALE	PREG.	IMPLANTS		EARLY DEATHS		LATE DEATHS		CORPORAL
				NO.	NO.		L	R	L	R	L	R	UTEA L H
TEM23	6	S	.0002	11	21	Y	6	6	2	0	1	1	6 6
TEM23	6	S	.0002	11	22	YY	4	8	0	2	0	0	5 5
TEM23	6	S	.0002	12	23	YY	0	8	0	0	0	0	4 8
TEM23	6	S	.0002	12	24	NN	5	7	0	0	-0	-0	5 7
TEM23	6	S	.0002	13	25	NN	-0	-0	-0	-0	-0	-0	-0 -0
TEM23	6	S	.0002	13	26	NN	-0	-0	-0	-0	-0	-0	-0 -0
TEM23	6	S	.0002	14	27	NN	-0	-0	-0	-0	-0	-0	-0 -0
TEM23	6	S	.0002	14	28	YY	5	8	0	0	0	0	4 8
TEM23	6	S	.0002	15	29	YY	8	3	1	1	1	1	4 3
TEM23	6	S	.0002	15	30	YY	4	7	0	0	1	0	4 7
TEM23	6	S	.0002	16	31	YY	5	6	0	0	3	0	5 6
TEM23	6	S	.0002	16	32	YY	6	7	0	0	0	1	7 8
TEM23	6	S	.0002	17	33	YY	5	8	0	0	0	0	5 8
TEM23	6	S	.0002	17	34	YY	2	3	0	0	0	0	4 8
TEM23	6	S	.0002	18	35	YY	7	4	0	0	0	0	7 6
TEM23	6	S	.0002	18	36	YY	10	0	2	0	0	0	11 2
TEM23	6	S	.0002	19	37	YY	5	8	-0	-0	-0	-0	5 8
TEM23	6	S	.0002	19	38	NY	-0	-0	-0	-0	-0	-0	-0 -0
TEM23	6	S	.0002	20	39	YY	7	5	0	0	1	3	7 6
TEM23	6	S	.0002	20	40	YN	-0	-0	-0	-0	-0	-0	-0 -0
CNTRL23	6	M	0.0000	1	1	Y	0	2	0	0	0	0	5 6
CNTRL23	6	M	0.0000	1	2	YY	8	5	0	1	0	0	8 7
CNTRL23	6	M	0.0000	2	3	YY	6	6	0	0	0	0	6 7
CNTRL23	6	M	0.0000	2	4	YY	6	5	0	0	0	0	6 5
CNTRL23	6	M	0.0000	3	5	YY	8	6	0	0	0	0	8 6
CNTRL23	6	M	0.0000	3	6	YY	7	5	0	0	0	0	7 4
CNTRL23	6	M	0.0000	6	7	YY	8	4	0	0	0	0	8 4
CNTRL23	6	M	0.0000	6	7	YY	7	5	0	0	0	0	7 4
CNTRL23	6	M	0.0000	4	8	YY	6	6	0	0	0	0	6 6
CNTRL23	6	M	0.0000	5	9	YY	5	7	0	0	0	0	5 6
CNTRL23	6	M	0.0000	5	10	YY	6	5	0	0	0	0	6 5
CNTRL23	6	M	0.0000	6	11	YY	3	9	0	0	0	0	3 3
CNTRL23	6	M	0.0000	6	12	YY	1	10	0	0	0	0	1 10
CNTRL23	6	M	0.0000	7	13	YY	1	6	1	0	0	0	7 10
CNTRL23	6	M	0.0000	7	14	YY	2	9	0	0	0	0	3 9
CNTRL23	6	M	0.0000	8	15	YY	5	9	0	1	0	0	5 10
CNTRL23	6	M	0.0000	8	16	YY	9	4	0	0	0	0	9 4
CNTRL23	6	M	0.0000	9	17	YY	6	7	0	0	0	0	6 7
CNTRL23	6	M	0.0000	9	18	YY	6	4	1	0	0	0	7 8
CNTRL23	6	M	0.0000	10	19	YY	5	4	0	0	0	0	5 9
CNTRL23	6	M	0.0000	10	20	Y	3	10	0	0	0	0	1 11

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD+C RED NO. 2

PAGE 24

TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS		EARLY DEATHS		LATE DEATHS		CORPOHA LUTEA	
							L	R	L	R	L	R	L	R
71-23	6	M	.0300	41	81	Y	6	7	0	0	0	0	9	7
71-23	6	M	.0300	41	82	Y	9	6	0	0	4	4	11	12
71-23	6	M	.0300	42	83	Y	5	6	0	0	0	0	5	7
71-23	6	M	.0300	42	84	Y	6	6	0	0	2	0	6	6
71-23	6	M	.0300	43	85	Y	7	5	0	0	0	0	7	7
71-23	6	M	.0300	43	86	Y	3	4	0	0	0	1	4	11
71-23	6	M	.0300	44	87	Y	7	4	2	0	0	0	7	6
71-23	6	M	.0300	44	88	Y	5	10	0	0	0	0	5	10
71-23	6	M	.0300	45	89	Y	6	8	0	0	1	0	7	8
71-23	6	M	.0300	45	90	Y	2	4	0	0	0	0	3	12
71-23	6	M	.0300	46	91	Y	3	7	0	0	0	0	4	7
71-23	6	M	.0300	46	92	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	6	M	.0300	47	93	Y	6	7	0	1	0	0	6	7
71-23	6	M	.0300	47	94	Y	4	6	0	0	0	0	4	7
71-23	6	M	.0300	48	95	Y	5	7	0	0	0	0	5	8
71-23	6	M	.0300	48	96	Y	8	4	0	0	0	0	5	4
71-23	6	M	.0300	49	97	Y	5	7	0	0	0	1	5	7
71-23	6	M	.0300	49	98	Y	4	6	0	0	0	0	5	6
71-23	6	M	.0300	50	99	Y	7	7	0	0	0	0	7	7
71-23	6	M	.0300	50	100	Y	4	9	0	0	0	1	6	12
71-23	6	M	2.5000	51	101	Y	6	7	0	0	0	1	6	7
71-23	6	M	2.5000	51	102	Y	3	10	0	0	0	3	4	10
71-23	6	M	2.5000	52	103	Y	6	7	0	0	3	2	6	7
71-23	6	M	2.5000	52	104	Y	4	9	0	0	0	0	9	9
71-23	6	M	2.5000	53	105	Y	10	1	1	1	2	0	11	1
71-23	6	M	2.5000	53	106	Y	8	5	0	0	0	0	8	5
71-23	6	M	2.5000	54	107	Y	4	8	0	1	0	0	5	9
71-23	6	M	2.5000	54	108	Y	9	3	0	0	0	1	5	10
71-23	6	M	2.5000	55	109	Y	5	7	0	0	0	0	5	9
71-23	6	M	2.5000	55	110	Y	5	9	0	0	0	0	4	8
71-23	6	M	2.5000	56	111	Y	4	8	0	0	0	1	11	2
71-23	6	M	2.5000	56	112	Y	10	2	0	0	3	0	7	6
71-23	6	M	2.5000	57	113	Y	7	6	0	0	0	0	11	5
71-23	6	M	2.5000	57	114	Y	10	5	0	0	0	0	13	10
71-23	6	M	2.5000	58	115	Y	8	1	0	0	0	0	5	6
71-23	6	M	2.5000	58	116	Y	0	1	0	0	0	0	5	6
71-23	6	M	2.5000	59	117	Y	5	6	0	0	0	0	5	7
71-23	6	M	2.5000	59	118	Y	6	7	0	0	0	1	5	7
71-23	6	M	2.5000	60	119	Y	5	7	0	0	0	2	5	8
71-23	6	M	2.5000	60	120	Y	8	7	0	0	0	5	2	7

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD+C RED NO. 2

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TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS		EARLY DEATHS		LATE DEATHS		CORPORA LUTEA	
							L	R	L	R	L	R	L	R
71-23	6	M	5.00000	61	121	Y	4	7	0	1	0	0	4	7
71-23	6	M	5.00000	61	122	YY	6	8	0	0	0	0	6	8
71-23	6	M	5.00000	62	123	YY	5	8	0	0	0	0	5	8
71-23	6	M	5.00000	62	124	YY	9	4	0	0	1	0	9	4
71-23	6	M	5.00000	63	125	YY	8	5	0	0	0	0	8	6
71-23	6	M	5.00000	63	126	YY	7	6	0	0	0	1	9	7
71-23	6	M	5.00000	64	127	Y	6	6	0	0	0	0	6	7
71-23	5	M	5.00000	64	128	YY	5	10	0	0	0	1	6	10
71-23	6	M	5.00000	65	129	YY	7	4	0	0	0	0	8	6
71-23	6	M	5.00000	65	130	YY	5	6	0	0	1	1	6	6
71-23	6	M	5.00000	66	131	YY	6	6	0	1	0	0	9	8
71-23	6	M	5.00000	66	132	YY	6	9	0	0	1	0	6	9
71-23	5	M	5.00000	67	133	YY	11	4	0	0	0	0	11	4
71-23	6	M	5.00000	67	134	YY	0	1	0	0	0	0	5	4
71-23	6	M	5.00000	68	135	YY	7	5	0	0	0	0	7	5
71-23	6	M	5.00000	68	136	YY	5	0	0	0	0	0	5	7
71-23	6	M	5.00000	69	137	YY	3	11	0	1	0	0	3	11
71-23	6	M	5.00000	69	138	YY	4	5	0	0	0	0	6	6
71-23	6	M	5.00000	70	139	YY	6	7	0	0	0	0	6	7
71-23	6	M	5.00000	70	140	Y	7	6	0	0	0	0	7	6

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

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TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA	
								L	R	L	R	L	R
71-23	7	S	2.5000	61	121	Y	10	3	0	0	0	0	10 4
71-23	7	S	2.5000	61	122	Y	4	7	0	0	0	0	4 7
71-23	7	S	2.5000	62	123	N	-0	-0	-0	-0	-0	-0	-0 -0
71-23	7	S	2.5000	62	124	Y	6	5	0	0	0	0	9 11
71-23	7	S	2.5000	63	125	Y	1	11	0	3	0	0	1 11
71-23	7	S	2.5000	63	126	Y	7	4	0	0	1	0	7 4
71-23	7	S	2.5000	64	127	Y	3	10	0	0	0	0	3 10
71-23	7	S	2.5000	64	128	N	-0	-0	-0	-0	-0	-0	-0 -0
71-23	7	S	2.5000	65	129	Y	4	7	0	0	0	0	4 7
71-23	7	S	2.5000	65	130	Y	3	7	0	0	0	0	3 9
71-23	7	S	2.5000	66	131	Y	6	6	0	1	0	0	6 7
71-23	7	S	2.5000	66	132	Y	4	4	0	0	0	0	5 4
71-23	7	S	2.5000	67	133	Y	2	10	0	0	0	1	2 11
71-23	7	S	2.5000	67	134	Y	6	6	0	0	0	0	7 6
71-23	7	S	2.5000	68	135	Y	6	6	1	1	0	0	7 6
71-23	7	S	2.5000	68	136	Y	8	2	0	0	0	0	9 3
71-23	7	S	2.5000	69	137	Y	6	8	0	0	2	1	6 8
71-23	7	S	2.5000	69	138	Y	9	4	0	0	0	0	9 4
71-23	7	S	2.5000	70	139	Y	9	4	0	0	1	1	10 5
71-23	7	S	2.5000	70	140	Y	6	7	0	0	0	0	6 8
71-23	7	S	5.0000	71	141	Y	6	2	1	0	1	1	7 5
71-23	7	S	5.0000	71	142	Y	1	0	1	0	0	0	10 5
71-23	7	S	5.0000	72	143	Y	9	2	0	0	0	0	9 2
71-23	7	S	5.0000	72	144	Y	1	1	0	0	0	0	4 7
71-23	7	S	5.0000	73	145	Y	10	4	0	0	0	0	10 5
71-23	7	S	5.0000	73	146	Y	5	5	0	0	0	1	5 5
71-23	7	S	5.0000	74	147	Y	4	9	0	1	0	0	4 9
71-23	7	S	5.0000	74	148	Y	6	9	0	0	0	1	7 9
71-23	7	S	5.0000	75	149	Y	4	8	0	0	0	1	4 8
71-23	7	S	5.0000	75	150	Y	6	5	0	0	0	0	8 6
71-23	7	S	5.0000	76	151	Y	5	6	0	0	0	0	5 7
71-23	7	S	5.0000	76	152	Y	2	8	0	0	1	0	2 5
71-23	7	S	5.0000	77	153	Y	7	4	2	0	0	0	7 6
71-23	7	S	5.0000	77	154	Y	4	6	0	0	0	0	4 6
71-23	7	S	5.0000	78	155	Y	7	7	0	0	0	0	7 6
71-23	7	S	5.0000	78	156	Y	6	6	0	0	1	0	6 6
71-23	7	S	5.0000	79	157	Y	6	4	0	0	1	0	6 4
71-23	7	S	5.0000	79	158	Y	6	3	0	0	0	0	6 3
71-23	7	S	5.0000	80	159	Y	4	8	0	0	0	0	7 9
71-23	7	S	5.0000	80	160	Y	3	9	0	0	0	0	3 10

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD+C RED NO. 2

PAGE 33

TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA		
								L	R	L	R	L	R	
TEM23	7	S	.0002	11	21	Y	9	3	0	0	0	0	9	3
TEM23	7	S	.0002	11	22	Y	4	10	0	2	0	0	5	10
TEM23	7	S	.0002	12	23	Y	7	6	0	0	0	0	7	6
TEM23	7	S	.0002	12	24	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	7	S	.0002	13	25	Y	9	4	0	0	0	0	9	4
TEM23	7	S	.0002	13	26	Y	5	5	0	0	0	0	5	5
TEM23	7	S	.0002	14	27	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	7	S	.0002	14	28	Y	5	5	0	0	0	0	-0	-0
TEM23	7	S	.0002	15	29	Y	9	3	1	0	0	0	9	3
TEM23	7	S	.0002	15	30	Y	8	4	0	0	4	0	9	4
TEM23	7	S	.0002	16	31	Y	6	0	0	0	0	0	6	8
TEM23	7	S	.0002	16	32	Y	2	10	0	0	0	0	6	18
TEM23	7	S	.0002	17	33	Y	10	4	0	0	0	1	10	4
TEM23	7	S	.0002	17	34	Y	6	6	0	0	0	0	6	6
TEM23	7	S	.0002	18	35	Y	7	5	0	0	1	0	7	5
TEM23	7	S	.0002	18	36	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	7	S	.0002	19	37	Y	3	10	0	0	0	1	4	10
TEM23	7	S	.0002	19	38	Y	5	5	0	0	0	0	5	5
TEM23	7	S	.0002	20	39	Y	6	3	0	0	0	1	6	3
TEM23	7	S	.0002	20	40	Y	0	1	0	0	0	1	2	5
CNTRL23	7	M	0.0000	1	1	Y	8	7	1	0	0	0	9	7
CNTRL23	7	M	0.0000	1	2	Y	6	7	1	0	0	0	6	7
CNTRL23	7	M	0.0100	2	3	Y	4	10	0	0	1	0	4	10
CNTRL23	7	M	0.0500	2	4	Y	7	5	4	0	0	0	7	5
CNTRL23	7	M	0.0200	3	5	Y	7	6	0	0	3	1	7	6
CNTRL23	7	M	0.0300	3	6	Y	5	6	0	0	0	0	5	7
CNTRL23	7	M	0.0100	4	7	Y	7	4	1	0	2	2	3	10
CNTRL23	7	M	0.0000	4	8	Y	3	10	0	0	0	2	8	4
CNTRL23	7	M	0.0000	5	9	Y	8	4	0	0	1	1	-0	-0
CNTRL23	7	M	0.0100	5	10	N	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	7	M	0.0100	6	11	Y	4	9	0	0	0	0	4	9
CNTRL23	7	M	0.0000	6	12	Y	5	4	0	0	0	0	5	4
CNTRL23	7	M	0.0100	7	13	N	-0	-0	-0	-0	-0	-0	-0	-0
CNTRL23	7	M	0.0000	7	14	Y	5	7	0	0	0	0	5	7
CNTRL23	7	M	0.0000	8	15	Y	9	7	0	0	0	0	9	7
CNTRL23	7	M	0.0000	8	16	Y	3	9	0	2	0	0	3	9
CNTRL23	7	M	0.0100	9	17	Y	5	7	1	0	0	0	5	7
CNTRL23	7	M	0.0000	9	18	Y	4	2	0	0	0	0	4	3
CNTRL23	7	M	0.0000	10	19	Y	6	4	1	0	0	1	6	4
CNTRL23	7	M	0.0100	10	20	Y	0	0	1	0	0	0	0	0

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

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TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA		
								L	R	L	R	L	R	
71-23	7	M	.0300	41	81	Y	5	8	0	0	0	0	5	R
71-23	7	M	.0300	41	82	Y	4	8	0	0	0	1	4	H
71-23	7	M	.0300	42	83	Y	6	7	0	0	0	0	6	7
71-23	7	M	.0300	42	84	Y	5	5	0	1	0	0	5	6
71-23	7	M	.0300	43	85	Y	10	3	0	0	0	4	6	5
71-23	7	M	.0300	43	86	Y	6	5	0	0	0	0	7	8
71-23	7	M	.0300	44	87	Y	7	8	0	0	2	1	6	6
71-23	7	M	.0300	44	88	Y	6	6	0	1	0	0	4	4
71-23	7	M	.0300	45	89	Y	8	4	0	0	0	0	6	6
71-23	7	M	.0300	45	90	Y	6	6	0	1	0	0	6	6
71-23	7	M	.0300	46	91	Y	6	6	0	0	1	0	6	5
71-23	7	M	.0300	46	92	Y	1	0	0	0	0	0	8	4
71-23	7	M	.0300	47	93	Y	8	4	0	0	0	1	9	3
71-23	7	M	.0300	47	94	Y	9	3	0	0	0	1	6	8
71-23	7	M	.0300	48	95	Y	6	8	0	0	0	0	7	4
71-23	7	M	.0300	48	96	Y	7	4	0	0	0	0	7	5
71-23	7	M	.0300	49	97	Y	1	0	0	0	0	1	7	5
71-23	7	M	.0300	49	98	Y	6	5	0	0	0	0	6	5
71-23	7	M	.0300	50	99	Y	7	6	0	0	0	0	7	4
71-23	7	M	.0300	50	100	Y	7	4	0	0	0	0	7	4
71-23	7	M	2.5000	51	101	Y	4	6	0	0	0	0	4	H
71-23	7	M	2.5000	51	102	Y	5	8	0	0	0	0	5	H
71-23	7	M	2.5000	52	103	Y	9	4	0	0	0	0	9	4
71-23	7	M	2.5000	52	104	Y	4	7	0	0	0	0	4	3
71-23	7	M	2.5000	53	105	Y	8	3	0	0	0	0	8	3
71-23	7	M	2.5000	53	106	Y	6	5	0	0	0	0	6	5
71-23	7	M	2.5000	54	107	Y	6	7	0	0	0	0	7	6
71-23	7	M	2.5000	54	108	Y	7	6	1	0	0	0	7	4
71-23	7	M	2.5000	55	109	Y	7	4	0	1	0	0	7	8
71-23	7	M	2.5000	55	110	Y	8	4	0	1	0	0	6	6
71-23	7	M	2.5000	56	111	Y	6	6	0	0	0	0	7	7
71-23	7	M	2.5000	56	112	Y	7	7	0	0	0	0	7	8
71-23	7	M	2.5000	57	113	Y	4	8	0	0	0	0	6	8
71-23	7	M	2.5000	57	114	Y	6	8	1	0	0	0	9	3
71-23	7	M	2.5000	58	115	Y	8	9	0	1	0	0	4	9
71-23	7	M	2.5000	58	116	Y	4	9	0	1	0	0	3	5
71-23	7	M	2.5000	59	117	Y	3	9	0	0	0	0	7	5
71-23	7	M	2.5000	59	118	Y	7	5	0	0	0	0	10	6
71-23	7	M	2.5000	60	119	Y	5	9	0	0	0	0	5	5
71-23	7	M	2.5000	60	120	Y	5	6	0	0	0	0	0	6

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

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PAGE 35

TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA		
								L	R	L	R	L	R	
71-23	7	M	5.00000	61	121	Y	9	4	0	0	1	0	9	4
71-23	7	M	5.00000	61	122	Y	8	5	0	0	0	0	8	5
71-23	7	M	5.00000	62	123	YY	7	6	1	0	1	0	7	6
71-23	7	M	5.00000	62	124	YY	6	8	0	1	0	0	6	8
71-23	7	M	5.00000	63	125	YY	9	6	0	0	2	0	9	6
71-23	7	M	5.00000	63	126	YY	7	5	1	0	0	0	7	5
71-23	7	M	5.00000	64	127	YY	5	3	1	0	0	0	9	5
71-23	7	M	5.00000	64	128	YY	8	4	0	0	0	0	8	4
71-23	7	M	5.00000	65	129	YY	1	4	0	4	0	0	2	10
71-23	7	M	5.00000	65	130	YY	9	3	1	1	0	0	11	3
71-23	7	M	5.00000	66	131	YY	9	6	0	0	0	0	9	6
71-23	7	M	5.00000	66	132	YY	6	7	0	0	0	0	6	7
71-23	7	M	5.00000	67	133	YY	7	3	0	0	0	0	8	3
71-23	7	M	5.00000	67	134	YY	6	7	1	0	0	0	6	7
71-23	7	M	5.00000	68	135	YY	5	4	1	1	0	0	7	4
71-23	7	M	5.00000	68	136	YY	5	6	0	0	0	0	5	6
71-23	7	M	5.00000	69	137	YY	4	0	0	0	0	0	4	5
71-23	7	M	5.00000	69	138	YY	7	4	0	0	0	0	7	4
71-23	7	M	5.00000	70	139	YY	6	5	0	0	0	0	6	5
71-23	7	M	5.00000	70	140	Y	11	4	0	0	0	0	13	4

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

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PAGE 36

TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS	EARLY DEATHS		LATE DEATHS		CORPORA LUTEA		
								L	R	L	R	L	R	
CNTRL23	8	S	0.0000	1	1	Y	8	3	0	0	1	0	9	3
CNTRL23	8	S	0.0000	1	2	Y	9	3	0	0	1	0	9	3
CNTRL23	8	S	0.0000	2	3	Y	6	5	0	0	0	0	6	5
CNTRL23	8	S	0.0000	2	4	Y	3	9	0	0	0	0	3	9
CNTRL23	8	S	0.0000	3	5	Y	7	7	0	0	0	0	7	7
CNTRL23	8	S	0.0000	3	6	Y	2	10	0	0	0	0	3	10
CNTRL23	8	S	0.0000	4	7	Y	3	3	0	0	0	0	7	3
CNTRL23	8	S	0.0000	4	8	Y	3	8	0	0	0	0	5	8
CNTRL23	8	S	0.0000	5	9	Y	5	7	0	0	0	0	5	7
CNTRL23	8	S	0.0000	5	10	Y	6	6	0	0	1	0	6	6
CNTRL23	8	S	0.0000	6	11	Y	6	6	0	0	0	0	6	7
CNTRL23	8	S	0.0000	6	12	Y	5	6	1	0	0	0	6	6
CNTRL23	8	S	0.0000	7	13	Y	9	1	0	0	0	0	4	1
CNTRL23	8	S	0.0000	7	14	Y	3	10	0	0	0	0	3	10
CNTRL23	8	S	0.0000	8	15	Y	8	5	0	0	0	1	8	5
CNTRL23	8	S	0.0000	8	16	Y	6	6	1	0	0	0	6	7
CNTRL23	8	S	0.0000	9	17	Y	6	7	0	1	0	0	7	3
CNTRL23	8	S	0.0000	9	18	Y	7	3	1	0	0	0	6	3
CNTRL23	8	S	0.0000	10	19	Y	6	3	0	0	0	0	3	4
CNTRL23	8	S	0.0000	10	20	Y	8	4	0	0	0	0	3	4
71-23	8	S	.0300	51	101	Y	0	1	0	0	0	1	7	4
71-23	8	S	.0300	51	102	Y	8	5	0	0	0	0	9	5
71-23	8	S	.0300	52	103	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	8	S	.0300	52	104	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	8	S	.0300	53	105	Y	6	7	0	0	1	0	5	7
71-23	8	S	.0300	53	106	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	8	S	.0300	54	107	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	8	S	.0300	54	108	N	-0	-0	-0	-0	-0	-0	-0	-0
71-23	8	S	.0300	55	109	Y	4	8	3	3	0	3	4	8
71-23	8	S	.0300	55	110	Y	7	6	0	0	0	0	8	6
71-23	8	S	.0300	56	111	Y	5	7	0	0	0	0	5	9
71-23	8	S	.0300	56	112	Y	4	5	0	0	0	1	5	6
71-23	8	S	.0300	57	113	Y	4	10	0	0	0	0	4	10
71-23	8	S	.0300	57	114	Y	0	9	0	0	0	0	4	9
71-23	8	S	.0300	58	115	Y	11	4	1	0	0	0	11	4
71-23	8	S	.0300	58	116	Y	3	9	0	0	0	0	3	6
71-23	8	S	.0300	59	117	Y	7	6	1	0	0	0	7	7
71-23	8	S	.0300	59	118	Y	6	6	1	0	0	0	6	7
71-23	8	S	.0300	60	119	Y	10	4	0	0	0	0	10	4
71-23	8	S	.0300	60	120	Y	6	4	0	0	0	0	6	4

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD&C RED NO. 2

PAGE 37

TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS				EARLY DEATHS		LATE DEATHS		CORPORA LUTEA	
							L	R	L	R	L	R	L	R	L	R
71-23	8	S	2.5000	61	121	Y	7	6	0	0	2	1	7	6		
71-23	8	S	2.5000	61	122	Y	4	7	0	1	0	0	4	7		
71-23	8	S	2.5000	62	123	Y	7	7	0	2	0	0	4	8		
71-23	8	S	2.5000	62	124	Y	5	6	0	0	0	0	6	6		
71-23	8	S	2.5000	63	125	Y	7	5	0	0	1	0	8	5		
71-23	8	S	2.5000	63	126	Y	6	5	0	1	0	0	6	7		
71-23	8	S	2.5000	64	127	Y	2	2	0	0	0	0	6	6		
71-23	8	S	2.5000	64	128	Y	2	8	0	0	0	0	2	8		
71-23	8	S	2.5000	65	129	Y	6	6	0	0	0	0	6	6		
71-23	8	S	2.5000	65	130	Y	7	5	2	0	0	0	12	7		
71-23	8	S	2.5000	66	131	Y	1	2	0	1	0	0	7	3		
71-23	8	S	2.5000	66	132	Y	7	5	1	0	0	0	9	5		
71-23	8	S	2.5000	67	133	Y	6	5	0	0	1	0	6	5		
71-23	8	S	2.5000	67	134	Y	4	8	0	0	0	0	4	10		
71-23	8	S	2.5000	68	135	Y	7	5	1	1	0	0	8	5		
71-23	8	S	2.5000	68	136	Y	7	5	1	0	0	0	8	5		
71-23	8	S	2.5000	69	137	Y	4	9	0	0	0	0	4	9		
71-23	8	S	2.5000	69	138	Y	8	5	1	0	0	0	8	5		
71-23	8	S	2.5000	70	139	Y	4	5	0	0	0	0	5	6		
71-23	8	S	2.5000	70	140	Y	3	11	1	1	0	0	2	3	11	
71-23	8	S	5.0000	71	141	Y	5	5	0	1	0	0	5	7		
71-23	8	S	5.0000	71	142	Y	9	2	0	0	0	0	4	5		
71-23	8	S	5.0000	72	143	Y	7	7	0	0	2	3	7	6		
71-23	8	S	5.0000	72	144	Y	5	6	0	0	0	0	5	6		
71-23	8	S	5.0000	73	145	Y	3	6	0	0	0	0	3	6		
71-23	8	S	5.0000	73	146	Y	8	4	1	0	0	0	3	4		
71-23	8	S	5.0000	74	147	Y	3	12	0	1	0	0	3	12		
71-23	8	S	5.0000	74	148	Y	5	6	1	0	0	0	5	6		
71-23	8	S	5.0000	75	149	Y	7	3	0	0	0	0	7	3		
71-23	8	S	5.0000	75	150	Y	4	6	1	0	0	0	4	7		
71-23	8	S	5.0000	76	151	Y	7	6	1	0	1	2	7	6		
71-23	8	S	5.0000	76	152	Y	3	9	0	0	0	0	3	9		
71-23	8	S	5.0000	77	153	Y	5	9	0	0	0	0	5	9		
71-23	8	S	5.0000	77	154	Y	5	3	0	0	0	0	5	7		
71-23	8	S	5.0000	78	155	Y	7	4	1	0	0	0	7	5		
71-23	8	S	5.0000	78	156	Y	8	4	0	0	0	0	8	4		
71-23	8	S	5.0000	79	157	Y	6	5	0	0	0	0	7	5		
71-23	8	S	5.0000	79	158	Y	6	6	0	0	0	0	6	6		
71-23	8	S	5.0000	80	159	Y	4	6	0	0	0	0	6	6		
71-23	8	S	5.0000	80	160	Y	5	8	0	0	0	0	5	8		

DOMINANT LETHAL GENE STUDY OF COMPOUND 71-23

FD+C RED NO. ?

PAGE 3B

TEST MATERIAL	WEEK	S/M	DOSE	MALE NO.	FEMALE NO.	PREG.	IMPLANTS		EARLY DEATHS		LATE DEATHS		CORPORA LUTEA	
							L	R	L	R	L	R	L	R
TEM23	8	S	.0002	11	21	Y	5	7	1	0	0	0	5	8
TEM23	8	S	.0002	11	22	Y	10	3	0	0	0	0	10	3
TEM23	8	S	.0002	12	23	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	8	S	.0002	12	24	Y	6	5	0	0	0	0	6	5
TEM23	8	S	.0002	13	25	Y	6	7	0	0	0	1	6	7
TEM23	8	S	.0002	13	26	Y	5	4	0	0	0	0	5	4
TEM23	8	S	.0002	14	27	Y	4	8	0	0	0	0	4	8
TEM23	8	S	.0002	14	28	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	8	S	.0102	15	29	Y	8	6	0	0	0	1	8	6
TEM23	8	S	.0002	15	30	Y	9	5	1	0	0	0	9	5
TEM23	8	S	.0002	16	31	Y	5	8	0	0	0	0	5	8
TEM23	8	S	.0002	16	32	Y	5	7	2	1	2	2	5	7
TEM23	8	S	.0002	17	33	Y	5	5	1	1	0	0	5	6
TEM23	8	S	.0002	17	34	Y	8	4	0	0	0	0	8	5
TEM23	8	S	.0002	18	35	Y	6	6	0	0	0	0	6	6
TEM23	8	S	.0002	18	36	Y	3	8	0	0	0	0	3	8
TEM23	8	S	.0102	19	37	Y	4	7	0	0	0	0	4	7
TEM23	8	S	.0002	19	38	Y	4	1	0	0	0	1	7	6
TEM23	8	S	.0002	20	39	N	-0	-0	-0	-0	-0	-0	-0	-0
TEM23	8	S	.0002	20	40	Y	1	0	1	0	0	0	5	6

CHI-SQUARE TEST OF THE FERTILITY INDEX (1 DEGREE OF FREEDOM)

WEEK	VEHICLE CONTROL				71-23 30 MG/KG				71-23 2.5 G/KG				71-23 5.0 G/KG				TEM 2.4G/KG			
	N PRG	N MTD	FERT. INDEX	CHISQ	N PRG	N MTD	FERT. INDEX	CHISQ	N PRG	N MTD	FERT. INDEX	CHISQ	N PRG	N MTD	FERT. INDEX	CHISQ	N PRG	N MTD	FERT. INDEX	CHISQ
SINGLE TREATMENT																				
1	16	20	.80	0.00	16	20	.80	.16	19	20	.95	.91	18	20	.90	.20	15	20	.75	0.00
2	16	20	.80	0.00	12	20	.60	1.07	16	20	.80	.16	18	20	.90	.20	15	20	.75	0.00
3	17	20	.85	0.00	14	20	.70	.57	16	20	.80	0.00	15	20	.75	.16	14	20	.90	0.00
4	18	20	.90	0.00	17	20	.85	0.00	18	20	.90	.28	16	20	.80	.20	15	20	.75	.69
5	20	20	1.00	0.00	16	20	.80	2.50	20	20	1.00	0.00	19	20	.95	0.00	17	20	.85	1.44
6	20	20	1.00	0.00	13	20	.65	6.23	17	20	.85	1.44	20	20	1.00	0.00	15	20	.75	3.66
7	20	20	1.00	0.00	15	20	.75	3.66	18	20	.90	.53	20	20	1.00	0.00	16	20	.80	2.50
8	20	20	1.00	0.00	15	20	.75	3.66	20	20	1.00	0.00	20	20	1.00	0.00	17	20	.85	1.44
MULTIPLE TREATMENT																				
1	16	20	.80	0.00	12	20	.60	1.07	12	20	.60	1.07	17	20	.65	0.00				
2	17	20	.85	0.00	18	20	.90	0.00	18	20	.90	0.00	17	20	.85	.20				
3	17	20	.85	0.00	17	20	.85	.20	17	20	.85	.20	16	20	.80	0.00				
4	17	20	.85	0.00	20	20	1.00	1.44	19	20	.95	.28	20	20	1.00	1.44				
5	20	20	1.00	0.00	19	20	.95	0.00	20	20	1.00	0.00	20	20	1.00	0.00				
6	20	20	1.00	0.00	19	20	.95	0.00	20	20	1.00	0.00	20	20	1.00	0.00				
7	18	20	.90	0.00	20	20	1.00	.53	20	20	1.00	.53	20	20	1.00	.53				

**ARMITAGE TEST FOR A LINEAR TREND IN PROPORTIONS FOR THE FERTILITY INDEX
(1 DEGREE OF FREEDOM) BASED ON THE DOSE LEVELS**

WEEK	30 MG/KG		2.5 G/KG		5.0 G/KG		CHISQ (C-1)	CHISQ (1)	ARMITG CHISQ
	N	PRG	N	PRG	N	PRG			
	MTD	MTD	MTD	MTD	MTD	MTD			
SINGLE TREATMENT									
1	16	20	19	20	18	20	2.26	.96	1.30
2	12	20	16	20	18	20	5.22	5.02	.19
3	14	20	16	20	15	20	.53	.13	.40
4	17	20	18	20	16	20	.78	.20	.59
5	16	20	20	20	19	20	5.67	2.93	2.75
6	13	20	17	20	20	20	8.88	8.81	.07
7	15	20	18	20	20	20	6.15	6.06	.09
8	15	20	20	20	20	20	10.91	8.15	2.76
MULTIPLE TREATMENT									
1	12	20	12	20	17	20	3.85	2.90	.95
2	18	20	18	20	17	20	.32	.24	.08
3	17	20	17	20	16	20	.24	.18	.06
4	20	20	19	20	20	20	2.03	.00	2.03
5	19	20	20	20	20	20	2.03	1.52	.51
6	19	20	20	20	20	20	2.03	1.52	.51
7	20	20	20	20	20	20	0.00	0.00	0.00

ARMITAGE TEST FOR A LINEAR TREND IN PROPORTIONS FOR THE FERTILITY INDEX
 (1 DEGREE OF FREEDOM) BASED ON THE LOGARITHMS OF THE DOSE LEVELS

WEEK	3.0 MG/KG			2.5 G/KG			5.0 G/KG			CHISQ (C-1)	CHISQ (1)	ARMTG
	N	N	PRG	N	N	PRG	N	N	PRG			
	PRG	MTD	---	PRG	MTD	---	PRG	MTD	---	---	---	---
SINGLE TREATMENT												
1	16	20		19	20		18	20	2.26	1.92	.44	
2	12	20		16	20		18	20	5.22	4.99	.22	
3	14	20		16	20		15	20	.53	.34	.19	
4	17	20		18	20		16	20	.78	.01	.77	
5	16	20		20	20		19	20	5.67	4.94	.73	
6	13	20		17	20		20	20	8.88	8.02	.86	
7	15	20		18	20		20	20	6.15	5.66	.48	
8	15	20		20	20		20	20	10.91	10.74	.17	
MULTIPLE TREATMENT												
1	12	20		12	20		17	20	3.85	1.41	2.45	
2	18	20		18	20		17	20	.32	.12	.21	
3	17	20		17	20		16	20	.24	.09	.15	
4	20	20		19	20		20	20	2.03	.31	1.73	
5	19	20		20	20		20	20	2.03	2.00	.03	
6	19	20		20	20		20	20	2.03	2.00	.03	
7	20	20		20	20		20	20	0.00	0.00	0.00	

ARMITAGE TEST FOR A LINEAR TREND IN PROPORTIONS FOR THE FERTILITY INDEX
(2 DEGREES OF FREEDOM) BASED ON THE DOSE LEVELS AND INCLUDING THE COVIRUL GROUP

CONTROL			30 MG/KG			2.5 G/KG			5.0 G/KG		
WEEK	N PRG	N MTD	N PRG	N MTD	N PRG	N MTD	N PRG	N MTD	CHISQ (C-1)	CHISQ (1)	ARMTG CHISQ
SINGLE TREATMENT											
1	16	20	16	20	19	20	18	20	2.85	1.61	1.23
2	16	20	12	20	16	20	18	20	5.45	3.13	2.32
3	17	20	14	20	16	20	15	20	1.43	.03	1.41
4	18	20	17	20	18	20	16	20	1.16	.47	.69
5	20	20	16	20	20	20	19	20	9.17	.92	8.25
6	20	20	13	20	17	20	20	20	15.09	3.31	11.78
7	20	20	15	20	18	20	20	20	10.49	2.36	8.13
8	20	20	15	20	20	20	20	20	16.00	4.29	11.71
MULTIPLE TREATMENT											
1	16	20	12	20	12	20	17	20	5.06	.93	4.14
2	17	20	18	20	18	20	17	20	.46	.04	.42
3	17	20	17	20	17	20	16	20	.28	.21	.07
4	17	20	20	20	19	20	20	20	6.32	1.56	4.76
5	20	20	19	20	20	20	20	20	3.04	.81	2.22
6	20	20	19	20	20	20	20	20	3.04	.81	2.22
7	18	20	20	20	20	20	20	20	6.15	1.70	4.45

T-TEST OF THE NUMBER OF IMPLANTATIONS IN PREGNANT FEMALES.

WEEK	CONTROL				71-23 30 MG/KG				71-23 2.5 G/KG				71-23 5.0 G/KG				TEM .2 MG/KG						
	N PRG	MEAN	STD DEV	N PRG	MEAN	STD DEV	DF	T	N PRG	MEAN	STD DEV	DF	T	N PRG	MEAN	STD DEV	DF	T	N PRG	MEAN	STD DEV	DF	T
SINGLE TREATMENT																							
1	16	12.62	1.89	16	12.12	3.22	30	.535	19	13.16	1.38	33	.960	18	12.00	1.94	32	.948	15	12.20	3.61	29	.914
2	16	12.44	1.55	12	12.50	1.45	26	.108	16	10.56	2.97	30	2.242	18	11.17	3.09	32	1.485	15	8.27	3.13	29	4.753
3	17	13.18	2.01	14	12.00	2.11	29	1.586	16	12.50	1.83	31	1.011	15	11.60	1.18	30	2.658	18	9.94	4.52	33	2.707
4	18	12.67	2.03	17	11.94	3.44	33	.766	18	12.67	1.50	34	0.000	16	10.75	3.73	32	1.889	15	4.93	2.66	31	9.476
5	20	11.65	1.42	16	13.56	1.90	34	3.457	20	11.50	2.89	38	.208	19	11.89	3.33	37	.301	17	10.94	4.66	35	.697
6	20	11.70	1.98	13	10.23	4.28	31	1.338	17	11.18	2.32	35	.741	20	11.65	2.41	38	.072	15	10.93	2.81	33	.948
7	20	11.15	2.35	15	11.40	3.31	33	.262	18	11.72	1.45	36	.893	20	10.40	3.50	38	.796	16	10.94	3.34	34	.224
8	20	11.40	1.73	15	11.47	3.38	33	.076	20	11.05	2.86	38	.469	20	11.00	2.75	38	.550	17	10.88	3.31	35	.609
MULTIPLE TREATMENT																							
1	16	13.13	1.31	12	10.33	2.81	26	3.516	12	11.17	2.89	26	2.413	17	10.00	4.14	31	2.885					
2	17	13.00	1.46	18	13.06	1.89	33	.097	18	13.72	1.36	33	1.514	17	13.53	3.00	32	.654					
3	17	12.76	2.70	17	12.65	2.85	32	.123	17	12.71	2.69	32	.064	16	11.75	3.32	31	.966					
4	17	13.06	1.39	20	12.25	2.61	35	1.144	19	12.58	3.20	34	.571	20	11.70	2.49	35	1.995					
5	20	12.30	1.78	19	11.95	2.12	37	.564	20	11.40	2.09	38	1.467	20	12.30	2.60	38	0.000					
6	20	11.50	2.56	19	11.68	2.38	37	.232	20	11.95	2.91	38	.519	20	11.80	3.38	38	.316					
7	18	11.94	2.24	20	11.05	3.62	36	.904	20	12.15	1.18	36	.360	20	11.45	3.03	36	.566					

REGRESSION FITS OF THE NUMBER, U, OF IMPLANTATIONS ON 1) DOSE, AND 2) LOG DOSE.
(PREDICTED U = A + BX) CONTROL GROUP EXCLUDED

WEEK	X	N	YBAR	SD X	UBAR	SD U	B	A	TB	DF	VARU,X	CV U	VARB	VARA	VARU,BAR
SINGLE TREATMENT															
1	DOSE	53	2.60	2.01	12.45	2.27	-.035	12.545	-.223	51	5.2719	.1844	.0252	.2700	.0995
	LOG DOSE	53	-.18	2.23	12.45	2.27	.068	12.465	.477	51	5.2537	.1841	.0204	.0998	.0991
2	DOSE	46	2.83	2.00	11.30	2.76	-.231	11.958	-1.124	44	7.5941	.2438	.0421	.5028	.1651
	LOG DOSE	46	-.23	2.15	11.30	2.76	-.314	11.315	-1.669	44	7.3471	.2398	.0354	.1598	.1597
3	DOSE	45	2.56	2.02	12.04	1.74	-.095	12.261	-.644	43	3.0045	.1458	.0172	.1819	.0685
	LOG DOSE	45	-.23	2.25	12.04	1.74	-.008	12.043	-.071	43	3.0138	.1465	.0140	.0699	.0692
4	DOSE	51	2.46	2.02	11.82	3.06	-.235	12.402	-1.100	49	9.3093	.2581	.0457	.4594	.1825
	LOG DOSE	51	-.34	2.28	11.82	3.06	-.080	11.796	-.418	49	9.5052	.2608	.0366	.1906	.1864
5	DOSE	55	2.65	2.00	12.24	2.90	-.319	13.080	-1.639	53	8.1516	.2333	.0379	.4132	.1482
	LOG DOSE	55	-.13	2.20	12.24	2.90	-.376	12.187	-2.167	53	7.8676	.2292	.0301	.1436	.1430
6	DOSE	50	2.46	2.01	11.12	2.97	.278	10.325	1.326	48	8.7076	.2654	.0440	.5335	.1742
	LOG DOSE	50	-.14	2.15	11.12	2.97	.259	11.109	1.318	48	8.7112	.2654	.0386	.1743	.1742
7	DOSE	53	2.74	2.03	11.13	2.91	-.218	11.731	-1.097	51	8.4299	.2608	.0395	.4568	.1591
	LOG DOSE	53	-.07	2.20	11.13	2.91	-.111	11.124	-.602	51	8.5680	.2629	.0341	.1618	.1617
8	DOSE	55	2.74	1.99	11.15	2.92	-.090	11.391	-.446	53	8.6625	.2641	.0406	.4612	.1575
	LOG DOSE	55	-.14	2.16	11.15	2.92	-.092	11.142	-.499	53	8.6544	.2640	.0342	.1574	.1574
MULTIPLE TREATMENTS															
1	DOSE	41	2.81	2.09	10.44	3.40	-.089	10.688	-.341	39	11.6134	.3293	.0673	.8212	.2881
	LOG DOSE	41	-.09	2.24	10.44	3.40	.002	10.439	.009	39	11.8486	.3297	.0589	.2895	.2890
2	DOSE	53	2.46	2.04	13.43	2.15	.097	13.196	.656	51	4.6863	.1611	.0217	.2200	.0884
	LOG DOSE	53	-.36	2.29	13.43	2.15	.114	13.475	.876	51	4.6558	.1606	.0170	.0901	.0878
3	DOSE	50	2.46	2.04	12.38	2.93	-.179	12.820	-.870	48	8.6098	.2370	.0423	.4281	.1722
	LOG DOSE	50	-.37	2.29	12.38	2.93	-.105	12.342	-.572	48	8.6663	.2381	.0337	.1782	.1737
4	DOSE	59	2.51	2.06	12.17	2.76	-.111	12.448	-.631	57	7.6711	.2276	.0311	.3257	.1300
	LOG DOSE	59	-.35	2.30	12.17	2.76	-.044	12.154	-.280	57	7.7140	.2282	.0252	.1338	.1307
5	DOSE	59	2.55	2.04	11.88	2.27	.074	11.692	.502	57	5.2430	.1927	.0218	.2307	.0889
	LOG DOSE	59	-.27	2.27	11.88	2.27	.001	11.882	.006	57	5.2661	.1931	.0177	.0906	.0893
6	DOSE	59	2.55	2.04	11.81	2.88	.022	11.756	.120	57	8.4356	.2459	.0350	.3712	.1430
	LOG DOSE	59	-.27	2.27	11.81	2.88	.036	11.823	.213	57	8.4310	.2458	.0293	.1450	.1429
7	DOSE	60	2.51	2.05	11.55	2.80	.080	11.350	.444	58	7.9531	.2442	.0322	.3354	.1326
	LOG DOSE	60	-.33	2.29	11.55	2.80	.139	11.595	.870	58	7.8775	.2430	.0256	.1340	.1313

REGRESSION FITS OF THE NUMBER, U, OF IMPLANTATIONS ON DOSE.
(PREDICTED U = A + B*X)
CONTROL GROUP INCLUDED

WEEK	X	N	XBAR	SD X	UBAR	SD U	B	A	TB	DF	VARU,X	CV U	VARB	VARA	VARU,BAR
SINGLE TREATMENT															
1	DOSE	69	2.00	2.08	12.49	2.18	-.044	12.581	-.344	67	4.8161	.1757	.0164	.1356	.0698
2	DOSE	62	2.10	2.13	11.60	2.54	-.289	12.205	-1.929	60	6.1978	.2147	.0225	.1993	.1000
3	DOSE	62	1.86	2.07	12.35	1.87	-.196	12.719	-1.712	60	3.4035	.1493	.0131	.1002	.0549
4	DOSE	69	1.82	2.04	12.04	2.84	-.266	12.527	-1.597	67	7.8630	.2328	.0277	.2055	.1140
5	DOSE	75	1.94	2.07	12.08	2.59	-.164	12.360	-.993	73	6.7246	.2147	.0212	.1693	.0897
6	DOSE	70	2.04	2.14	11.29	2.72	.100	11.082	.646	68	7.4876	.2425	.0238	.2061	.1070
7	DOSE	73	1.99	2.12	11.14	2.75	-.146	11.429	-.956	71	7.5734	.2471	.0235	.1969	.1037
8	DOSE	75	2.01	2.09	11.21	2.65	-.091	11.396	-.615	73	7.0673	.2371	.0219	.1822	.0942
MULTIPLE TREATMENTS															
1	DOSE	57	2.02	2.18	11.19	3.19	-.385	11.971	-2.020	55	9.6629	.2777	.0363	.3180	.1695
2	DOSE	70	1.86	2.06	13.33	2.01	.118	13.109	1.007	68	4.0200	.1504	.0137	.1050	.0574
3	DOSE	67	1.84	2.06	12.48	2.86	-.173	12.795	-1.012	65	8.1593	.2289	.0291	.2199	.1218
4	DOSE	76	1.95	2.10	12.37	2.53	-.172	12.704	-1.240	74	6.3767	.2042	.0193	.1572	.0839
5	DOSE	79	1.91	2.08	11.99	2.16	.006	11.977	.047	77	4.7140	.1811	.0139	.1103	.0597
6	DOSE	79	1.91	2.08	11.73	2.79	.051	11.636	.336	77	7.8770	.2392	.0233	.1844	.0997
7	DOSE	78	1.93	2.08	11.64	2.67	.018	11.606	.122	76	7.2348	.2311	.0216	.1734	.0928

T-TEST TEST OF THE (TRANSFORMED) PRE-IMPLANTATION LOSSES IN PREGNANT FEMALES.
(LOSSES TAKEN AS A SUBSET OF THE SET OF CORPORA LUTEA)

WEEK	CONTROL		71-23 30 MG/KG				71-23 2.5 G/KG				71-23 5.0 G/KG				TEM		.2 MG/KG										
	N	PRG	MEAN	STD DEV	N	PRG	MEAN	STD DEV	DF	T	N	PHG	MEAN	STD DEV	DF	T	N	PRG	MEAN	STD DEV	DF	T	N	PRG	MEAN	STD DEV	DF
SINGLE TREATMENT																											
1	16	.58	.29	16	.69	.50	30	.744	19	.58	.21	33	.005	18	.63	.38	32	.441	15	.85	.55	29	1.742				
2	16	.56	.25	12	.58	.37	26	.224	16	.83	.54	30	1.824	18	.70	.55	32	.923	15	1.21	.51	29	4.542				
3	17	.45	.33	14	.72	.44	29	1.985	16	.59	.44	31	1.041	15	.51	.28	30	.587	18	1.05	.77	33	2.956				
4	18	.53	.35	17	.68	.61	33	.917	18	.54	.25	34	.068	16	.92	.60	32	2.357	15	1.81	.38	31	10.115				
5	20	.62	.31	16	.52	.31	34	.965	20	.53	.47	38	.696	19	.63	.54	37	.060	17	.94	.70	35	1.869				
6	20	.47	.28	13	.83	.77	31	1.926	17	.64	.40	35	1.573	20	.59	.42	38	1.087	15	.66	.56	33	1.318				
7	20	.63	.43	15	.72	.53	33	.527	18	.60	.32	36	.302	20	.72	.64	36	.503	16	.64	.63	34	.054				
8	20	.47	.29	15	.64	.59	33	1.186	20	.72	.51	38	1.916	20	.56	.46	38	.803	17	.57	.61	35	.055				
MULTIPLE TREATMENT																											
1	16	.52	.23	12	.78	.54	26	1.707	12	.60	.54	26	.549	17	.86	.71	31	1.451									
2	17	.46	.22	18	.49	.43	33	.282	18	.55	.24	33	1.178	17	.53	.50	32	.587									
3	17	.63	.42	17	.64	.49	32	.040	17	.60	.42	32	.196	16	.54	.54	31	.544									
4	17	.49	.28	20	.60	.45	35	.866	19	.68	.54	34	1.262	20	.53	.43	35	.284									
5	20	.54	.24	19	.61	.41	37	.672	20	.54	.38	38	.029	20	.44	.34	38	1.106									
6	20	.71	.50	19	.75	.45	37	.239	20	.64	.58	38	.425	20	.67	.57	38	.245									
7	18	.39	.21	20	.52	.66	36	.795	20	.37	.18	36	.316	20	.58	.50	36	1.549									

T-TEST OF THE (TRANSFORMED) NUMBER OF DEAD IMPLANTS.

WEEK	CONTROL				71-23 30 MG/KG				71-23 2.5 G/KG				71-23 5.0 G/KG				TEM .2 MG/KG						
	N PRG	MEAN	STD DEV	N PRG	MEAN	STD DEV	DF	T	N PRG	MEAN	STD DEV	DF	T	N PRG	MEAN	STD DEV	DF	T	N PRG	MEAN	STD DEV	DF	T
SINGLE TREATMENT																							
1	16	.56	.31	16	.45	.20	30	1.142	19	.66	.42	33	.809	18	.45	.21	32	1.240	15	1.16	.45	29	4.342
2	16	.46	.23	12	.57	.35	26	1.005	16	.42	.20	30	.493	18	.46	.46	32	.031	15	1.70	.49	29	9.091
3	17	.47	.24	14	.52	.31	29	.428	16	.41	.21	31	.749	15	.44	.25	30	.415	18	1.70	.61	33	7.740
4	18	.56	.25	17	.60	.36	33	.451	18	.58	.37	34	.209	16	.56	.33	32	.078	15	1.27	.34	31	6.910
5	20	.47	.29	16	.55	.29	34	.840	20	.49	.28	38	.250	19	.47	.23	37	.073	17	1.11	.45	35	5.299
6	20	.40	.19	13	.55	.31	31	1.699	17	.52	.33	35	1.410	20	.39	.18	38	.159	15	.75	.37	33	3.666
7	20	.46	.23	15	.49	.35	33	.379	18	.49	.30	36	.390	20	.53	.26	38	.964	16	.53	.30	34	.843
8	20	.46	.24	15	.55	.46	33	.690	20	.62	.29	38	1.837	20	.52	.31	38	.649	17	.54	.37	35	.758
MULTIPLE TREATMENT																							
1	16	.56	.35	12	.53	.28	26	.303	12	.54	.42	26	.156	17	.53	.30	31	.333					
2	17	.53	.32	18	.46	.19	33	.765	18	.63	.39	33	.814	17	.49	.31	32	.350					
3	17	.62	.37	17	.41	.26	32	1.916	17	.49	.32	32	1.071	16	.48	.24	31	1.309					
4	17	.44	.24	20	.50	.20	35	.792	19	.50	.25	34	.710	20	.45	.36	35	.074					
5	20	.46	.26	19	.39	.18	37	1.060	20	.52	.30	38	.670	20	.56	.39	38	.962					
6	20	.48	.28	19	.48	.29	37	.058	20	.59	.44	38	.996	20	.43	.21	38	.612					
7	18	.68	.38	20	.52	.32	36	1.363	20	.40	.18	36	2.935	20	.54	.30	36	1.233					

CHI-SQUARE TEST OF THE DEATH INDEX (1 DEGREE OF FREEDOM)

WEEK	VEHICLE CONTROL				71-23 30 MG/KG				71-23 2.5 G/KG				71-23 5.0 G/KG				TEM .2 MG/KG			
	N WDI	N PRG	DEATH INDEX	CHISQ	N WDI	N PRG	DEATH INDEX	CHISQ	N WDI	N PRG	DEATH INDEX	CHISQ	N WDI	N PRG	DEATH INDEX	CHISQ	N WDI	N PRG	DEATH INDEX	CHISQ
SINGLE TREATMENT																				
1	9	16	.56	0.90	8	16	.50	0.00	14	19	.74	.53	8	18	.44	.12	14	15	.93	3.79
2	7	16	.44	0.00	7	12	.58	.15	6	16	.38	0.00	4	18	.22	.94	15	15	1.00	9.32
3	8	17	.47	0.00	7	14	.50	.04	6	16	.38	.04	5	15	.33	.18	18	18	1.00	10.21
4	11	18	.61	0.00	10	17	.59	.04	9	18	.50	.11	9	16	.56	0.00	15	15	1.00	5.26
5	7	20	.35	0.00	9	16	.56	.48	8	20	.40	0.00	9	19	.47	.21	17	17	1.00	14.30
6	6	20	.30	0.00	7	13	.54	1.01	7	17	.41	.13	6	20	.30	.12	11	15	.73	4.83
7	8	20	.40	0.00	5	15	.33	0.0	7	18	.39	.07	11	20	.55	.40	8	16	.50	.07
8	8	20	.40	0.00	7	15	.47	0.0	13	20	.63	1.60	9	20	.45	0.00	8	17	.47	.01
MULTIPLE TREATMENT																				
1	9	16	.56	0.80	6	12	.50	0.0	5	12	.42	.15	8	17	.47	.03				
2	9	17	.53	0.00	10	18	.56	.03	10	18	.56	.03	7	17	.41	.12				
3	11	17	.65	0.00	5	17	.29	2.95	7	17	.41	1.06	7	16	.44	.74				
4	7	17	.41	0.00	12	20	.60	.66	10	19	.53	.12	6	20	.30	.13				
5	8	20	.40	0.00	6	19	.32	.05	9	20	.45	0.00	9	20	.45	0.00				
6	8	20	.40	0.00	8	19	.42	.04	9	20	.45	0.00	8	20	.40	.10				
7	11	18	.61	0.00	9	20	.45	.45	6	20	.30	2.56	10	20	.50	.13				

ARMITAGE TEST FOR A LINEAR TREND IN PROPORTIONS FOR THE DEATH INDEX
(1 DEGREE OF FREEDOM)
BASED ON THE DOSE LEVELS

WEEK	30 MG/KG			2.5 G/KG			5.0 G/KG			CHISQ (C-1)	CHISQ (1)	ARMTG CHISQ
	N	N	WDI	N	N	WDI	N	N	WDI	PRG	PRG	PRG
	WDI	PRG	---	---	---	---	---	---	---	---	---	---
SINGLE TREATMENT												
1	8	16		14	19		8	18	3.62	.16	3.46	
2	7	12		6	16		4	18	4.03	4.00	.04	
3	7	14		6	16		5	15	.90	.83	.08	
4	10	17		9	18		9	16	.29	.02	.27	
5	9	16		8	20		9	19	.94	.23	.71	
6	7	13		7	17		6	20	1.88	1.88	.00	
7	5	15		7	18		11	20	1.86	1.73	.13	
8	7	15		13	20		9	20	1.91	.05	1.86	
MULTIPLE TREATMENT												
1	6	12		5	12		8	17	.17	.01	.16	
2	10	18		10	18		7	17	.96	.71	.24	
3	5	17		7	17		7	16	.83	.73	.10	
4	12	20		10	19		6	20	3.91	3.62	.29	
5	6	19		9	20		9	20	.96	.71	.25	
6	8	19		9	20		8	20	.10	.02	.08	
7	9	20		6	20		10	20	1.78	.11	1.68	

ARMITAGE TEST FOR A LINEAR TREND IN PROPORTIONS FOR THE DEATH INDEX
 (1 DEGREE OF FREEDOM)
 BASED ON THE LOGARITHMS OF THE DOSE LEVELS

	3.0 MG/KG		2.5 G/KG		5.0 G/KG				
WEEK	N WDI	N PRG	N WDI	N PRG	N WDI	N PRG	CHISQ (C-1)	CHISQ (1)	ARMTG CHISQ
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SINGLE TREATMENT									
1	8	16	14	19	8	18	3.62	.16	3.47
2	7	12	6	16	4	18	4.03	3.59	.44
3	7	14	6	16	5	15	.90	.89	.01
4	10	17	9	18	9	16	.29	.12	.17
5	9	16	8	20	9	19	.94	.62	.33
6	7	13	7	17	6	20	1.88	1.61	.27
7	5	15	7	18	11	20	1.86	1.11	.75
8	7	15	13	20	9	20	1.91	.14	1.77
MULTIPLE TREATMENT									
1	6	12	5	12	8	17	.17	.07	.10
2	10	18	10	18	7	17	.96	.34	.62
3	5	17	7	17	7	16	.83	.83	.00
4	12	20	10	19	6	20	3.91	2.39	1.52
5	6	19	9	20	9	20	.96	.95	.02
6	8	19	9	20	8	20	.10	.00	.10
7	9	20	6	20	10	20	1.78	.04	1.74

ARMITAGE TEST FOR A LINEAR TREND IN PROPORTIONS FOR THE DEATH INDEX
 (2 DEGREES OF FREEDOM)
 BASED ON THE DOSE LEVELS AND INCLUDING THE CONTROL GROUP

WEEK	CONTROL		30 MG/KG		2.5 G/KG		5.0 G/KG		CHISQ (C-1)	CHISQ (1)	ARMTG CHISQ
	N	N	N	N	N	N	WDI	PRG			
	WDI	PRG	WDI	PRG	WDI	PRG	WDI	PRG			
SINGLE TREATMENT											
1	9	16	8	16	14	19	8	18	3.62	.10	3.52
2	7	16	7	12	6	16	4	18	4.19	3.55	.64
3	8	17	7	14	6	16	5	15	1.14	1.06	.08
4	11	18	10	17	9	18	9	16	.50	.14	.36
5	7	20	9	16	8	20	9	19	1.85	.02	1.83
6	6	20	7	13	7	17	6	20	2.55	.39	2.15
7	8	20	5	15	7	18	11	20	1.94	1.50	.44
8	8	20	7	15	13	20	9	20	2.85	.15	2.70
MULTIPLE TREATMENT											
1	9	16	6	12	5	12	8	17	.62	.24	.39
2	9	17	10	18	10	18	7	17	.98	.64	.34
3	11	17	5	17	7	17	7	16	4.45	.09	4.36
4	7	17	12	20	10	19	6	20	4.13	2.01	2.12
5	8	20	6	19	9	20	9	20	.97	.55	.42
6	8	20	8	19	9	20	8	20	.14	.00	.14
7	11	18	9	20	6	20	10	20	3.85	.22	3.63

PROBIT ANALYSIS OF THE PROPORTION OF PREGNANT FEMALES WITH 1 OR MORE DEAD IMPLANTS
 PROBIT = A + B(LOG DOSE)

WEEK	B	A	CHISQ	DF
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SINGLE TREATMENT

1	.071	5.172	3.50	1
2	-.383	4.658	.54	1
3	-.183	4.725	.02	1
4	-.063	5.114	.17	1
5	-.140	4.923	.33	1
6	-.244	4.746	.29	1
7	.198	4.836	.73	1
8	.067	5.070	1.78	1

MULTIPLE TREATMENT

1	-.053	4.906	.10	1
2	-.102	5.008	.62	1
3	.170	4.717	.00	1
4	-.258	4.896	1.58	1
5	.168	4.780	.02	1
6	-.002	4.807	.10	1
7	-.034	4.785	1.75	1

T-TEST OF THE (TRANSFORMED) NUMBER OF DEAD IMPLANTS.
(DEAD IMPLANTS TAKEN AS A SUBSET OF THE SET OF IMPLANTS)

WEEK	CONTROL				71-23 30 MG/KG				71-23 2.5 G/KG				71-23 5.0 G/KG				TEM .2 MG/KG						
	N PRG	STD MEAN	STD DEV	N PHG	N MEAN	STD DEV	DF	T	N PHG	STD MEAN	STD DEV	DF	T	N PRG	STD MEAN	STD DEV	DF	T	N PRG	STD MEAN	STD DEV	DF	T
SINGLE TREATMENT																							
1	16	.58	.33	16	.50	.25	30	.773	19	.64	.45	33	.777	18	.48	.24	32	1.048	15	1.36	.51	29	5.149
2	16	.48	.24	12	.59	.35	26	1.006	16	.48	.22	30	.025	18	.53	.61	32	.314	15	2.38	.52	29	13.193
3	17	.49	.27	14	.55	.31	29	.523	16	.44	.21	31	.695	15	.45	.26	30	.456	18	2.23	.41	33	14.657
4	18	.57	.26	17	.68	.39	33	.932	18	.60	.39	34	.239	16	.73	.62	32	.976	15	2.46	.30	31	19.391
5	20	.49	.30	16	.57	.29	34	.805	20	.52	.29	38	.338	19	.51	.25	37	.309	17	1.40	.56	35	6.278
6	20	.41	.20	13	.74	.59	31	2.287	17	.55	.34	35	1.525	20	.42	.20	38	.058	15	.81	.36	33	4.115
7	20	.48	.23	15	.54	.37	33	.632	18	.51	.30	36	.335	20	.66	.69	38	1.471	16	.64	.54	34	1.232
8	20	.47	.24	15	.67	.65	33	1.240	20	.68	.32	38	2.285	20	.55	.31	38	.834	17	.66	.59	35	1.327
MULTIPLE TREATMENT																							
1	16	.58	.35	12	.62	.39	26	.270	12	.62	.55	26	.268	17	.67	.52	31	.591					
2	17	.54	.33	18	.49	.20	33	.614	18	.65	.39	33	.859	17	.52	.31	32	.248					
3	17	.67	.40	17	.44	.28	32	1.875	17	.52	.32	32	1.215	16	.51	.24	31	1.382					
4	17	.45	.24	20	.53	.21	35	1.056	19	.61	.49	34	1.178	20	.47	.36	35	.171					
5	20	.48	.26	19	.41	.19	37	.884	20	.55	.33	38	.830	20	.57	.38	38	.944					
6	20	.52	.29	19	.54	.36	37	.178	20	.64	.44	38	1.005	20	.47	.22	38	.553					
7	18	.68	.39	20	.63	.52	36	.352	20	.40	.19	36	2.928	20	.61	.44	36	.553					

CONTROL GROUP ANOVA FOR THE NUMBER OF PREGNANT FEMALES

WEEK	BETWEEN MALES			WITHIN MALES			TOTAL			F
	SUMSQ	DF	MEANSQ	SUMSQ	DF	MEANSQ	SUMSQ	DF		
SINGLE TREATMENT										
1	1.200	9	.133	2.000	10	.200	3.200	19	.667	
2	1.200	9	.133	2.000	10	.200	3.200	19	.667	
3	1.050	9	.117	1.500	10	.150	2.550	19	.778	
4	.800	9	.089	1.000	10	.100	1.800	19	.889	
5	0.000	9	0.000	0.000	10	0.000	0.000	19	I	
6	0.000	9	0.000	0.000	10	0.000	0.000	19	I	
7	0.000	9	0.000	0.000	10	0.000	0.000	19	I	
8	0.000	9	0.000	0.000	10	0.000	0.000	19	I	
MULTIPLE TREATMENT										
1	2.200	9	.244	1.000	10	.100	3.200	19	2.444	
2	1.050	9	.117	1.500	10	.150	2.550	19	.778	
3	1.050	9	.117	1.500	10	.150	2.550	19	.778	
4	2.050	9	.228	.500	10	.050	2.550	19	4.556	
5	0.000	9	0.000	0.000	10	0.000	0.000	19	I	
6	0.000	9	0.000	0.000	10	0.000	0.000	19	I	
7	.800	9	.089	1.000	10	.100	1.800	19	.889	

CONTROL GROUP ANOVA FOR THE NUMBER OF IMPLANTATIONS PER PREGNANT FEMALE

WEEK	BETWEEN MALES			WITHIN MALES			TOTAL			F
	SUMSQ	DF	MEANSQ	SUMSQ	DF	MEANSQ	SUMSQ	DF		
SINGLE TREATMENT										
1	17.260	9	1.918	36.500	6	6.083	53.760	15	.315	
2	32.160	9	3.573	4.500	6	.750	36.660	15	4.764	
3	42.062	9	4.674	22.500	7	3.214	64.562	16	1.454	
4	47.345	9	5.261	23.500	8	2.938	70.845	17	1.791	
5	23.050	9	2.561	15.500	10	1.550	38.550	19	1.652	
6	27.200	9	3.022	47.000	10	4.700	74.200	19	.643	
7	45.050	9	5.006	59.500	10	5.950	104.550	19	.841	
8	26.800	9	2.978	30.000	10	3.000	56.800	19	.993	
MULTIPLE TREATMENT										
1	9.278	8	1.160	16.500	7	2.357	25.778	15	.492	
2	20.542	9	2.282	13.500	7	1.929	34.042	16	1.184	
3	64.080	9	7.120	53.000	7	7.571	117.080	16	.940	
4	17.441	8	2.180	13.500	8	1.688	30.941	16	1.292	
5	25.200	9	2.800	35.000	10	3.500	60.200	19	.800	
6	46.000	9	5.111	79.000	10	7.900	125.000	19	.647	
7	52.445	9	5.827	32.500	8	4.063	84.945	17	1.434	

CONTROL GROUP ANOVA FOR THE PRE-IMPLANTATION LOSS PER PREGNANT FEMALE

WEEK	BETWEEN MALES			WITHIN MALES			TOTAL			F
	SUMSQ	DF	MEANSQ	SUMSQ	DF	MEANSQ	SUMSQ	DF		
SINGLE TREATMENT										
1	43.360	9	4.818	28.000	6	4.667	71.360	15	1.032	
2	2.840	9	.316	9.000	6	1.500	11.840	15	.210	
3	17.082	9	1.898	20.500	7	2.929	37.582	16	.648	
4	25.905	9	2.878	12.500	8	1.563	38.405	17	1.842	
5	16.050	9	1.783	14.500	10	1.450	30.550	19	1.230	
6	6.450	9	.717	8.500	10	.850	14.950	19	.843	
7	24.200	9	2.689	30.000	10	3.000	54.200	19	.896	
8	15.450	9	1.717	3.500	10	.350	18.950	19	4.905	
MULTIPLE TREATMENT										
1	2.512	8	.314	6.500	7	.929	9.012	15	.338	
2	1.743	9	.194	4.500	7	.643	6.243	16	.301	
3	39.330	9	4.370	31.000	7	4.429	70.330	16	.987	
4	17.306	8	2.163	28.500	8	3.563	45.806	16	.607	
5	7.200	9	.800	6.000	10	.600	13.200	19	1.333	
6	89.800	9	9.978	46.000	10	4.600	135.800	19	2.169	
7	2.125	9	.236	3.500	8	.438	5.625	17	.540	

CONTROL GROUP ANOVA FOR THE NUMBER OF DEAD IMPLANTS PER PREGNANT FEMALE

WEEK	BETWEEN MALES			WITHIN MALES			TOTAL			F
	SUMSQ	DF	MEANSQ	SUMSQ	DF	MEANSQ	SUMSQ	DF		
SINGLE TREATMENT										
1	15.000	9	1.667	4.000	6	.667	19.000	15	2.500	
2	4.569	9	.507	3.500	6	.583	8.060	15	.869	
3	5.930	9	.659	4.000	7	.571	9.930	16	1.153	
4	5.780	9	.642	6.000	8	.750	11.780	17	.856	
5	6.800	9	.756	10.000	10	1.000	16.800	19	.756	
6	3.200	9	.356	1.000	10	.100	4.200	19	3.556	
7	3.000	9	.333	6.000	10	.600	9.000	19	.556	
8	8.000	9	.889	3.000	10	.300	11.000	19	2.963	
MULTIPLE TREATMENT										
1	19.327	8	2.416	28.500	7	4.071	47.427	15	.593	
2	10.282	9	1.142	17.500	7	2.500	27.782	16	.457	
3	34.082	9	3.787	22.500	7	3.214	56.582	16	1.178	
4	5.750	8	.719	4.500	8	.563	10.250	16	1.278	
5	4.450	9	.494	6.500	10	.650	10.950	19	.761	
6	6.200	9	.689	14.000	10	1.400	20.200	19	.492	
7	20.805	9	2.312	21.500	8	2.688	42.305	17	.360	

CONTROL GROUP ANOVA FOR THE RATIO OF DEAD IMPLANTS TO TOTAL IMPLANTS PER PREGNANT FEMALE

WEEK	BETWEEN MALES			WITHIN MALES			TOTAL			F
	SUMSQ	DF	MEANSQ	SUMSQ	DF	MEANSQ	SUMSQ	DF		
SINGLE TREATMENT										
1	.119	9	.013	.040	6	.007	.159	15		2.014
2	.019	9	.002	.024	6	.004	.042	15		.519
3	.033	9	.004	.043	7	.006	.076	16		.598
4	.031	9	.003	.040	8	.005	.072	17		.683
5	.049	9	.005	.081	10	.008	.130	19		.673
6	.028	9	.003	.008	10	.001	.036	19		3.708
7	.020	9	.002	.038	10	.004	.058	19		.574
8	.058	9	.006	.021	10	.002	.078	14		3.130
MULTIPLE TREATMENT										
1	.086	8	.011	.130	7	.019	.218	19		.592
2	.072	9	.008	.120	7	.017	.192	16		.465
3	.186	9	.021	.135	7	.019	.321	16		1.073
4	.034	8	.004	.027	8	.003	.061	19		1.274
5	.036	9	.004	.046	10	.005	.082	19		.360
6	.043	9	.005	.091	10	.009	.134	19		.529
7	.137	9	.015	.164	8	.021	.301	17		.741

T-TEST OF THE NUMBER OF CORPORA LUTEA IN PREGNANT FEMALES.

WEEK	CONTROL				71-23 30 MG/KG				71-23 2.5 G/KG				71-23 5.0 G/KG				TEM .2 MG/KG					
	N PRG	MEAN	STD DEV	N PRG	MEAN	STD DEV	DF	T	N PRG	MEAN	STD DEV	DF	T	N PRG	MEAN	STD DEV	DF	T	N PRG	MEAN	STD DEV	DF

SINGLE TREATMENT

1	16	13.87	3.46	16	13.94	1.91	30	.063	19	14.11	1.15	33	.273	18	13.39	1.50	32	.542	15	15.27	3.39	29	1.430
2	16	13.31	1.40	12	13.92	2.57	26	.797	16	13.06	2.41	30	.359	18	13.00	1.94	32	.932	15	12.53	2.07	29	1.236
3	17	13.88	1.27	14	14.21	3.49	29	.365	16	14.12	2.42	31	.364	15	12.33	1.18	30	3.566	18	15.50	6.30	33	1.038
4	18	13.67	1.81	17	13.94	1.64	33	.469	18	13.50	1.50	34	.300	16	13.56	2.00	32	.152	15	12.80	1.61	31	1.436
5	20	12.80	1.15	16	14.62	2.31	34	3.097	20	12.25	1.83	38	1.137	19	13.47	2.34	37	1.149	17	14.18	1.78	35	2.828
6	20	12.25	1.59	13	12.69	2.53	31	.619	17	12.41	1.23	35	.342	20	12.80	1.47	38	1.137	15	12.60	1.12	33	.728
7	20	12.45	1.50	15	12.93	2.22	33	.769	18	12.94	2.26	36	.801	20	12.40	2.11	38	.086	16	12.75	3.66	34	.334
8	20	11.95	1.32	15	12.93	1.33	33	2.174	20	12.85	2.06	38	1.647	20	11.95	1.54	38	0.000	17	12.12	1.32	35	.386

MULTIPLE TREATMENT

1	16	13.87	1.02	12	12.33	1.07	26	3.862	12	12.50	1.09	26	3.424	17	12.47	1.70	31	2.851
2	17	13.53	1.33	18	14.33	2.00	33	1.392	18	14.67	1.28	33	2.576	17	14.94	1.71	32	2.685
3	17	14.18	1.47	17	14.35	1.37	32	.363	17	14.06	1.64	32	.221	16	13.00	1.90	31	1.999
4	17	13.94	2.28	20	13.65	1.84	35	.430	19	14.47	2.57	34	.655	20	12.80	1.94	35	1.648
5	20	13.10	1.89	19	13.42	2.61	37	.442	20	12.30	1.17	38	1.608	20	12.85	1.95	38	.411
6	20	13.40	2.04	19	13.95	2.90	37	.686	20	13.85	2.74	38	.590	20	13.40	1.88	38	0.000
7	18	12.22	2.16	21	12.15	1.18	36	.130	20	12.40	1.19	36	.319	20	12.70	1.84	36	.737